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9258 P

Effect of Artificial Achylia Gastrica and a Diet Restricted in Vitamin B_2 (G) on Hematopoiesis.

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A mechanism implicated in the production of Addisonian (pernicious; macrocytic) anemia, is demonstrated by Castle[1] in the inability of patients to digest properly and obtain from food an antianemic principle in amounts adequate to maintain a normal number of erythrocytes in the circulating blood. As a result of these investigations, many attempts have been made to produce in laboratory animals a disease syndrome similar to that of pernicious anemia.

Ivy, Morgan and Farrell[2] have reported the occasional occurrence of a spontaneous anemia in gastrectomized dogs, and have also noted an increased tendency towards anemia during pregnancy in their

* Research assistant by a grant from the Christine Breon Fund for Medical Research.

1 Castle, W. B., *Am. J. Med. Sc.*, 1929, **178**, 748.

2 Ivy, A. C., Morgan, J. E., and Farrell, J. I., *Surg., Gyn. and Obst.*, 1931, **53**, 611.

gastrectomized dogs. Gutzeit,[3] Aron and Bauer,[4] and Maison and Ivy,[5] in studies on rats and dogs found that a similar anemia occurred following the surgical removal of the animals' stomachs. Mullenix, Dragstedt and Bradley[6] reported that their dogs after gastrectomy, showed a reduced capacity to form hemoglobin. Mettier, Kellogg and Purviance[7] produced an hypochromic anemia in their dogs by frequent bleeding; and observed, after the surgical removal of the animals' stomachs, that the blood showed a more marked microcytosis.

Miller and Rhoades[8, 9] produced what they interpreted as a macrocytic anemia in dogs and in swine by using a diet deficient in vitamin B.

The present problem, therefore, was undertaken to study blood formation in gastrectomized dogs while on a diet low in vitamin B_2 (G).

Three mongrel German shepherd dogs, 2 males (No. 393 and No. 161) and one female (No. 473), were used for this study. These animals had been gastrectomized at least one year prior to the present experiment, for the purpose of making studies on hypochromic anemia, the results of which have been published elsewhere.[7]

The diet used for the experiment was similar to that used by Miller and Rhoades.[9]

Preceding this study, analysis of the upper intestinal contents of all 3 dogs showed absence of free hydrochloric acid even after the subcutaneous administration of histamine.

After approximately 80 days had elapsed, the animals showed the symptom-complex characteristic of black-tongue. Diarrhea and progressive weakness developed; there was a slight loss of bodyweight; and marked stomatitis with salivation appeared.

During the control period the erythrocyte count of each of the 3 animals was slightly above 6,000,000 cells per cu.mm., and the hemoglobin content was between 60% (8.4 gm.) and 70% (9.8 gm.). The mean corpuscular volume, as shown in Table I, was 62 (No. 473), 50 (No. 393), and 66 (No. 161) cubic microns respectively. [Normal values for man (average): mean corpuscular volume, 87.0 cubic microns; mean corpuscular hemoglobin concentra-

[3] Gutzeit, K., *Verhandl. d. deutsch. Gesellschaft f. inn. Med.*, 1932, **44**, 478.

[4] Aron, E., and Bauer, R., *Compt. rend. Soc. de Biol.*, 1935, **113**, 1065.

[5] Maison, G. L., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 554.

[6] Mullenix, R. B., Dragstedt, C. A., and Bradley, J. D., *Am. J. Physiol.*, 1933, **105**, 443.

[7] Mettier, S. R., Kellogg, F., and Purviance, K., *J. Clin. Invest.* (In press.)

[8] Rhoades, C. P., and Miller, D. K., *J. Exp. Med.*, 1933, **58**, 585.

[9] Miller, D. K., and Rhoades, C. P., *J. Clin. Invest.*, 1935, **14**, 153.

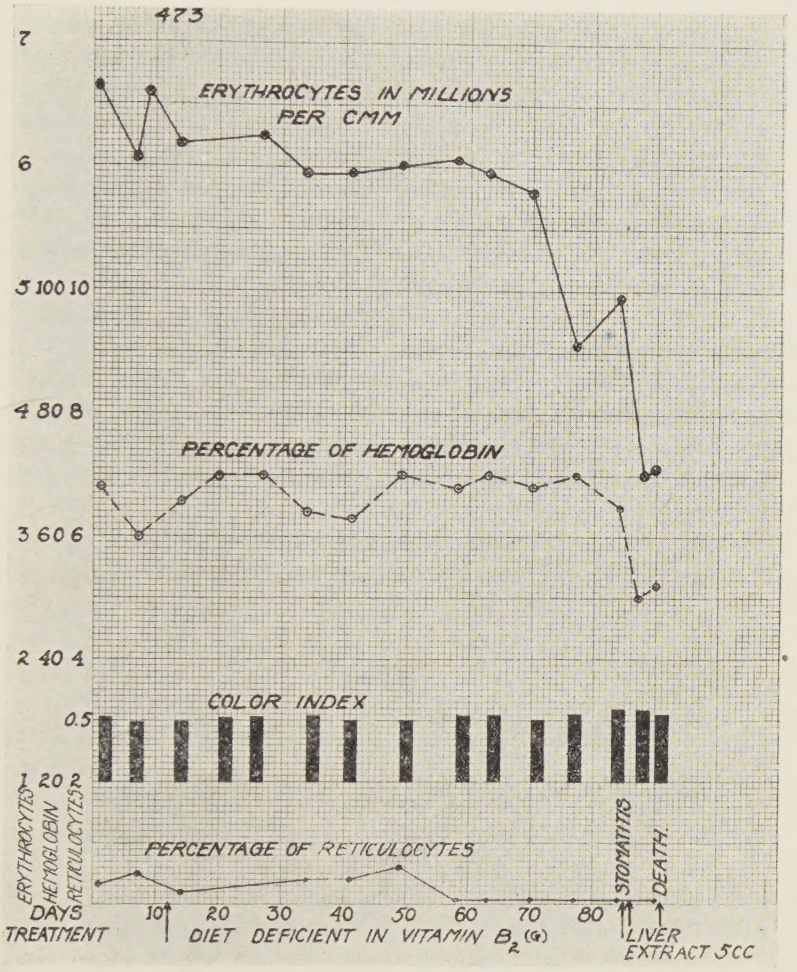

FIG. 1.

Shows a slight drop in the erythrocyte and hemoglobin levels in a gastrectomized dog (No. 475) during the first 15 days on a diet restricted in vitamin B_2 (G) and a more precipitous drop after the animal developed black-tongue. Note the slight terminal rise in color index but the persistence of hypochromic anemia.

tion, 35% (Wintrobe). For dogs: mean corpuscular volume 78.0 cubic microns; mean corpuscular hemoglobin concentration, 25.2% (unpublished data).]

During the first 15 days on the diet restricted in vitamin B_2 (G), there was an average drop of 1,250,000 in the number of red blood cells per cu.mm., and a slight decrease in hemoglobin. For the next 60 days the erythrocytes and their hemoglobin content remained at fairly constant levels.

The blood counts of 2 animals (No. 473 and No. 393) that were

continued on the diet until exitus, were noteworthy during the last 2 weeks of the experiment in that there was a precipitous drop in both the erythrocyte and hemoglobin levels. The final red blood-cell counts were 4,260,000 (No. 473) and 3,900,000 (No. 393) cells per cu.mm., and the hemoglobin determinations were 52 (7.4 gm.) and 36 (5 gm.) % respectively. It is to be emphasized that, throughout the experiment, the color indices remained between 0.5 and 0.7 and the cell-volume less than normal. The anemia, therefore, continued to be hypochromic and microcytic in character.

In these 2 dogs parenteral injections of liver extract† failed to alleviate symptoms, or to alter the progress of the anemia.

The third dog (No. 161) showed a reduction in the number of erythrocytes after 75 days of restricted diet. Progress of the anemia stopped, however, with the daily feeding of raw beef which is rich in vitamin B_2 (G), and subsequently there was a rise in the erythrocyte level.

Conclusion. A diet restricted in vitamin B_2 (G) when fed to dogs with an artificial achylia gastrica did not produce macrocytic anemia.

9259 P

Cortico-Adrenal Insufficiency in Rats Under Reduced Pressure.

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This report concerns one of the aspects of a broader research project in which a fairly comprehensive study was made of the response of rats to reduced pressure, inclusive of the relation of such response to some pathological conditions both in rats and other animals. By experiments carried out under a variety of standardized levels of low pressure and for a series of standardized periods of exposure to such pressures, it has been shown that various functional factors respond in a uniform and typical manner to these environments. On this evidence 3 stages could be distinguished with respect to the total exposure period of a rat to the low pressure environment: (a) a pre-adaptive stage during which most deviations of values indicate an unfavorable effect, (b) an adaptive stage during which deviations occur in the opposite direction and are indicative of adaptive adjustments, and (c) a post-adaptive stage during

† The authors wish to thank Eli Lilly and Co. for their generous contribution of Liver Extract (N.N.R.).

which functional shifts occur again in a direction suggesting pathological changes. The third of these stages was found to occur mainly when the animals were exposed for extended periods to very low levels of pressure and was considered to be an expression of an exhausted adaptive function.

It was considered also that the functional changes occurring during this third stage would be the most promising material for suggesting the nature of the general functional state which is produced by artificial and possibly also natural altitude conditions. For this purpose the total picture of physiological effects found during this stage was compared with the syndromes of various pathological conditions of known etiology. It was discovered that the functional alterations under these particular low pressure conditions are identical with the functional alterations resulting from cortico-adrenal insufficiency in adrenalectomized animals. Some earlier observations, *viz.*, the sudden and unexpected deaths of apparently normal rats and the finding of intestinal hemorrhages in many of these rats, supported the assumption that the adrenal activity is involved in the deterioration of animals in this abnormal "climate." It was reasoned that if some direct proof could be obtained for the correctness of this assumption it would become useful later for relating acclimatization to altitude with the function of the adrenal cortex. It was the purpose of this particular investigation to furnish such experimental proof.

If a deficiency of the cortical hormone is the direct cause of the abnormal functional effects during the post-adaptive stage it could be predicted that administration of such hormone would ameliorate the condition of the rats. This prediction was proven to be correct. The rats which received charcoal adsorbate of the cortical hormone with the food lived much longer than low pressure rats without cortin. Moreover, the administration of cortical extract had a definite normalizing effect on the various functional levels in the former group of rats.

If the cortico-adrenal activity is inhibited at later stages under reduced pressure it would be expected that some structural damage of the gland could be demonstrated to have occurred previously. Such histological evidence for adrenal damage was obtained. In cases in which this damage was not easily detectable from cellular necrosis and hemorrhages, it consisted of a confluence of lipoid droplets which Zwemer has postulated as a sign of cortico-adrenal insufficiency.

Of the several causes which could be suggested for this structural and functional damage an exhaustion due to overwork appeared

a priori to be the most likely. In order to substantiate this hypothesis it was necessary to provide a reasonable explanation for an initial stimulation of the cortical activity under reduced pressure. A greater demand for cortical hormone by the body under diminished pressure appeared as the most reasonable explanation. Attempts to obtain some evidence for an increase of the cortin requirements of low pressure rats constituted the third step in this investigation.

The cortin requirements of adrenalectomized rats were first carefully determined with respect to atmospheric conditions. This procedure was repeated with respect to a series of graded low pressure environments. The amounts of assayed charcoal adsorbates of cortical hormone needed to maintain the adrenalectomized low pressure rats alive increased progressively with the lowering of the pressure gradient. Moreover, the increment in rat units per 100 mm. decrease in pressure underwent a gradual and quite considerable rise with the lowering of the pressure. The minimum dose was more than 20 times higher at a pressure of 300 mm. than normally. It may be inferred that rats with intact adrenals under a similar pressure require an equal amount of cortical hormone and that their cortical apparatus may not be able to supply all of this demand without finally becoming exhausted.

9260

Relation of Certain Bile Acids to Absorption of β-Carotene in the Rat.*

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In previous work from this laboratory[1] it has been shown that deoxycholic acid acts as a carrier of irradiated ergosterol and of β-carotene across the intestinal tract of the rat. This is probably

* We are indebted to E. R. Squibb and Sons for the fellowship and for supplies of cod liver oil and vitamin A concentrate. Technical assistance was kindly supplied by the Works Progress Administration. We are indebted to Reidel-de Haen, Inc., for the supply of decholin.

† E. R. Squibb and Sons Fellow, 1934-37.

1 Greaves, J. D., and Schmidt, C. L. A., *J. Biol. Chem.*, 1933, **102**, 101; *Univ. of Calif. Pub. Physiol.*, 1934, **8**, 43, 49; *Am. J. Physiol.*, 1935, **111**, 492, 502; 1936, **116**, 456. Schmidt, C. L. A., *Pac. Coast Med.*, 1937, **4**, 16.

due to the formation of the respective choleic acids.[2] The present experiments were undertaken to determine whether or not taurocholic acid, glycocholic acid, and decholin can transport β-carotene in a manner similar to that of deoxycholic acid.

Two series of procedures were employed. In the first series vitamin A deficient bile fistula rats were fed a mixture of β-carotene and the bile acid under test. The vaginal smear picture was used as an index of absorption and utilization of β-carotene. Each animal received 100 γ of β-carotene daily during the test period. The activity of the carotene was controlled by feeding similar doses of the β-carotene-bile acid mixtures to unoperated vitamin A deficient rats. Thirteen bile fistula rats were employed for the glycocholic acid group, 12 for the taurocholic acid group, and 11 for the decholin group. With the exception of one rat in the glycocholic acid group none of the bile fistula rats responded to the administration of β-carotene. The vaginal smear pictures of the unoperated vitamin A deficient rats returned to normal. Under similar experimental conditions absorption of β-carotene takes place when deoxycholic acid is fed to bile fistula rats.

In the second series attempts were made to prepare compounds of β-carotene with each of the three bile acids under consideration. Ten milligram portions of β-carotene were mixed with 6 molecular equivalents of the bile acid. The mixtures were sealed in glass tubes under nitrogen and heated in a paraffin bath to 185-190° for 5 minutes. The melt was dissolved in an excess of 5% sodium carbonate solution. The filtered solutions were extracted with peroxide-free ether. Some of the solutions were previously acidified, others were not. The first part of the extract was discarded in order to remove any uncombined carotene. The remaining portions of the extracted material were evaporated to dryness in an atmosphere of nitrogen. The residue was dissolved in sodium carbonate solution and the process of extraction, evaporation, and solution was repeated. A similar experiment was carried out with deoxycholic acid.

With the exception of the deoxycholic acid-carotene preparation none of the other bile acid-carotene preparations gave a positive antimony trichloride test. The vaginal smear pictures of none of the vitamin A deficient rats, when fed the glycocholic, taurocholic, or decholin preparations, returned to normal, while a positive re-

[2] Shimizu, T., and Hatekeyama, T., *Z. physiol. Chem.*, 1929, **182**, 57; Yamasaki, K., *J. Biochem.* (Tokio), 1935, **22**, 243; Von Euler, H., and Klassman, E., *Z. physiol. Chem.*, 1933, **219**, 215.

sponse was shown by the animals which received the deoxycholic acid-carotene compound. Certain of the rats which failed to respond to the above mentioned 3 bile acid preparations showed normal vaginal smear pictures when the deoxycholic acid-carotene preparation was fed.

β-carotene, when administered intravenously either via the jugular or the portal vein, to certain animals is less effective than when it is administered by mouth.[3] The present experiments were carried out on rats. A hot saturated solution of β-carotene in acetone was poured into physiological saline solution under continuous agitation with nitrogen. The mixture was filtered and heated to remove traces of acetone. The resulting suspension was used for purposes of injection. Injections were likewise made of a suspension of a vitamin A concentrate in a gum arabic solution. This suspension was not very stable. The injections of β-carotene were made into the heart, into the carotid artery, and into the jugular and portal veins. β-carotene was also administered by means of the stomach tube and injected subcutaneously. The vitamin A suspensions were given by mouth and injections were also made into the heart and into the portal circulation. All injections were made under ether anesthesia. Doses of 0.5 to 1.0 mg. of β-carotene were administered. The doses of vitamin A were comparable.

The vaginal smear pictures of only about one-half of the rats which received injections of β-carotene into the blood stream returned to normal. The vaginal smears of these animals showed a continuous cornified cell picture within a week after the injections were made, indicating that the response to the β-carotene was of short duration. When β-carotene was injected subcutaneously most of the animals responded and the response was of somewhat longer duration than in the case of those animals in which the β-carotene was injected into the blood stream. The vaginal smears of all of the animals which received β-carotene orally became normal and continued so for at least 4 weeks.

The increase in weight of the animals which received injections of β-carotene was slight, whereas the rats which received β-carotene by mouth showed decided increases in weight. In all instances the rats which received vitamin A showed decided increases in weight and the vaginal smears returned to normal. Administration of vitamin A by mouth was found to be more effective than when the same dose was injected, although the difference was much less

[3] Rea, J. L., and Drummond, J. C., *Z. Vit. Forsch.*, 1932, **1**, 177; Drummond, J. C., and Macwalter, J., *Biochem. J.*, 1933, **27**, 1342; *J. Physiol.*, 1935, **83**, 236; Ahmad, B., Grewal, K. S., and Malik, K. S., *Ind. Med. Gaz.*, 1934, **69**, 320.

marked than in the case of β-carotene. The decreased effect of β-carotene, when injected into the blood stream, cannot be explained on the basis of passive filtration in the lungs since injections, when made directly into the portal system, were no more effective than when they were made into the heart or into the jugular vein.

Summary. No chemical evidence was obtained that taurocholic acid, glycocholic acid, and decholin form compounds with β-carotene. When β-carotene, together with taurocholic acid, glycocholic acid, or decholin, was fed to bile fistula vitamin A deficient rats, the β-carotene was not utilized. When β-carotene, in the form of a suspension, was injected intravenously, it was less effective than when administered orally. A less marked difference was noted in the case of vitamin A.

9261 P

Ultrafiltration of Psittacosis Virus.

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Levinthal[1] using the "gradocol" membranes of Elford, estimated the particle size of the virus of psittacosis to be 220 to 330mμ. Sir Henry Dale[2] has referred to unpublished experiments of Elford, in which the virus was found to be 275 mμ in diameter. No data are available on the details of filtration or the type of material used in these experiments. Microscopic measurements show the elementary body to range in size from 200 to 300 mμ,[3] while microphotographic studies give the smallest elementary bodies a diameter of 240 to 300 mμ.[4] In connection with psittacosis studies being conducted in this laboratory, it was considered of interest to determine the size of the infective particle under controlled conditions.

The virus strain used was isolated from infected shell parakeets in 1934 and has had no known connection with a human case. The virus was carried according to routine in white mice until December, 1935, when the strain was established on the chorio-allantoic mem-

* Edith Claypole Memorial Research Fellow in Pathology, 1936-37.

1 Levinthal, W., *Lancet*, 1935, **1**, 1207.

2 Dale, H. H., *Huxley Memorial Lecture*, 1935.

3 Lillie, R. D., *U. S. Pub. Health Rep.*, 1930, **45**, 773.

4 Coles, A. C., *Lancet*, 1930, **1**, 1011.

brane of the developing chick. Over 190 consecutive bacteria-free passages have been made to date, with the virus maintaining an unaltered infectiousness for susceptible white mice. All virus used for ultrafiltration studies was obtained from membranes inoculated subsequent to the 43rd passage.

The virus suspensions were prepared from 72-hour membranes. After aseptic removal from the egg, the infected tissue was placed in 50 cc. Erlenmeyer flasks containing 2 cc. of the indicated diluent per membrane. After agitating for 20 minutes in a mechanical shaker, the mixture was centrifuged for 20 minutes at 3000 RPM and the clear supernatant fluid removed for ultrafiltration. Control mice receiving 1 cc. of a 10^{-6} dilution of this material intraperitoneally succumbed in 9-12 days with typical findings for psittacosis. Routine staining of infected membranes and mouse organs was done by the original method of Castaneda,[5] and the presence of L.C.L. bodies was demonstrated in all cases.

Ultrafiltration was conducted at room temperature under a positive pressure of nitrogen; the method of Elford,[6] as modified by Bauer and Hughes[7] was used. Pressures varied from 18 to 30 cm. Hg, and the volume of filtrate ranged from 5 to 12 cc. All filtrations were complete in 4 minutes or less. Membrane thicknesses ranged from .136 to .152 mm. Use of a 1-100 dilution of the original supernatant fluid obviated the necessity of a preliminary filtration to remove gross tissue particles. *Chromobacterium prodigiosum* was added before filtration and was absent from filtrates in all cases. All membranes were satisfied by the preliminary passage of 5 cc. of the diluting fluid.

The infective agent passed readily through membranes with average pore diameters of 645, 454, 436 and 410 mμ; hormone broth pH 7.3 and buffered water† pH 7.3 were used as the suspending and diluting media. Filtrates showed practically no decrease in titer when inoculated mice were compared with those receiving unfiltered material. With one inconclusive exception, the virus was completely retained by membranes with an average pore diameter of 383 mμ or less.

On the basis of these results, the filtration end-point is approximately 400 mμ. By the application of Elford's correction factor for this range, the diameter of the infective agent of psittacosis is found to be 200 to 300 mμ, a figure in close agreement with microscopic and microphotographic measurements of the elementary body.

[5] Castaneda, M. R., *J. Infect. Dis.*, 1930, **47**, 416.

[6] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[7] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

† McIlvaine's standard, diluted 1-50.

9262 P

Age Factor and Latent Period in Production of Sarcoma by Methylcholanthrene in Rats.*

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It has been shown that the latent period for the production of epidermoid carcinoma in mice by tarring is not influenced by the age of the animals.[1] Recently Dunning, Curtis and Bullock[2] as a result of extensive observations on the latent period in the production of sarcoma by subcutaneous injections of dibenzanthracene and benzpyrene in rats and mice also concluded that the age of the injected animals was of no significance, although in some of the studies upon mice the latent period appeared to be definitely prolonged in younger subjects than in older ones.

Histologic study of the subcutaneous connective tissue in white rats at birth, at 2 to 3 weeks of age, at 2 to 3 months of age and in the adult animal reveal various stages in the maturation of this tissue. The following experiments were performed to observe, again, whether the age of the injected animal bore any relationship to the latent period for the development of sarcoma following the subcutaneous injection of a carcinogenic compound.

The animals used were all members of the same colony which has been inbred in this laboratory for the past 5 years, having originated from 3 males and 3 females procured from a dealer. One group of 13 young rats, injected at 3 weeks of age was compared with a group of 12 adults 1 to 2 years of age. The carcinogenic substance employed was methylcholanthrene. Single subcutaneous injections were made in the interscapular region and consisted of 2 mg. of the compound in 1/10 cc. lard.

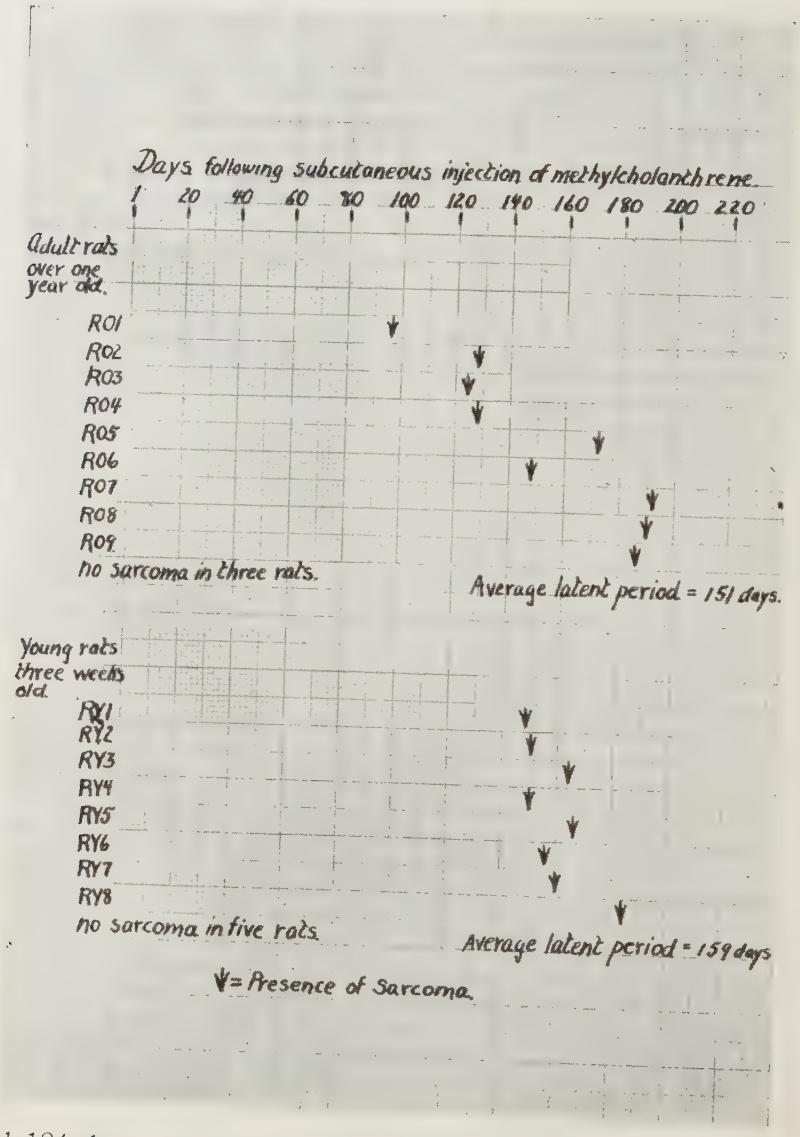
The presence of a tumor was recorded when it had reached about one cm. in diameter in order to avoid possible confusion with the injected mass. The results are summarized in Table I.

As shown, the shortest latent period was 96 days, the longest 192 days. Both instances were in the adult group. The latent periods among the young group varied between narrower limits, *i. e.*, 148

* This work was facilitated by a grant from the Cancer Research Institute of the Chicago Woman's Club.

1 Woglom, W. H., *Arch. Path.*, 1926, **1**, 533.

2 Dunning, W., Curtis, M. R., and Bullock, F. D., *Am. J. Cancer*, 1936, **28**, 681.



and 184 days. The average latent periods of the 2 groups were practically the same, 150 to 160 days. The experiment was terminated 220 days after the injections. Three animals did not develop tumors among the adult group and 5 had not developed sarcomas among the young group, during the period of observation.

The results indicate that maturing young, relatively less differentiated subcutaneous tissue in recently born and rapidly growing rats is not more prone to sarcomatous degeneration as a result of ex-

posure to a given carcinogenic agent than is adult subcutaneous tissue in the "static state" of a fully developed rat exposed to a similar dose of the same agent.

Thus, as in the case of epidermoid carcinoma produced by tar and sarcoma produced by the subcutaneous injection of dibenzanthracene and benzpyrene, the age of the animal bears little or no relationship to the latent period in the production of sarcoma by subcutaneous injection of methylcholanthrene.

9263

A Sensitive Method for Quantitative Estimation of Epinephrine in Blood.

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Meltzer and Auer[1] observed that epinephrine causes dilatation of the pupil much more readily after the corresponding superior cervical ganglion has been excised, in rabbits. By means of the "paradoxical" pupil reaction, Joseph and Meltzer[2] demonstrated liberation of epinephrine from the adrenals on stimulation of the splanchnic nerves. This was confirmed by Elliott,[3] who found that dilatation of the pupil and retraction of the nictitating membrane, resulting from electrical excitation of the splanchnic nerve, do not occur in the absence of the adrenals.

Following these qualitative observations, Stewart and Rogoff[4] employed the eye reactions (pupil dilatation, retraction of nictitating membrane and widening of palpebral aperture), sensitized by corresponding superior cervical ganglionectomy, for quantitative studies on the epinephrine output of the adrenals. A method of "autoassay" was devised based upon reactions of the sensitized eye when adrenal vein blood was collected in a "cava pocket" for a given time and then released into the circulation. The intensity of the epinephrine reactions was measured by intravenous injections of appropriate amounts of epinephrine.

In our experience with various biological test objects used for

* Aided by the G. N. Stewart Memorial Fund and a grant from the Rosenwald Family Association.

1 Meltzer, S. J., and Auer, C. M., *Am. J. Physiol.*, 1904, **11**, 28.

2 Joseph, D. R., and Meltzer, S. J., *Am. J. Physiol.*, (Proc. Am. Physiol. Soc.), 1912, **29**, xxxiv.

3 Elliott, T. R., *J. Physiol.*, 1912, **44**, 374.

4 Stewart, G. N., and Rogoff, J. M., *J. Pharm. Exp. Therap.*, 1916, **8**, 479.

detecting epinephrine, it was observed that sensitivity of the reagent usually increases in the course of an experiment. An increase of 5 to 25 or more times may occur after the test object has been used for an hour or two. This is quite common with segments of rabbit's intestine and uterus. In earlier work we observed increased sensitivity to epinephrine with the so-called "denervated" or sensitized eye reactions but not of such magnitude as was seen with intestine segments.

The present report will show that it is possible to increase sensitivity of the denervated eye to epinephrine to such a degree that the reactions can be utilized for detection of epinephrine in small amounts in blood-serum or in saline solutions. Quantitative estimation of the epinephrine concentration in the liquid can be made by comparing the reactions with those obtained by injection of epinephrine solutions of different concentrations. By this method it was found possible to detect, with certainty, epinephrine in serum or in physiological saline solution in dilutions of 1:100,000,000 to 1:500,000,000. Sometimes the sensitivity is not so great and occasionally an animal may yield reactions with even higher dilutions.

Procedure. The method depends upon further sensitizing the eye to epinephrine after it has already been sensitized by superior cervical ganglionectomy. This is accomplished by repeated injections of epinephrine in amounts sufficient to elicit small but definite reactions. Cats were used 5 or 6 days to 2 weeks after unilateral excision of the superior cervical ganglion. Sensitive reactions have been obtained up to about a month after ganglionectomy. The animal is anesthetized (urethane, 0.75 to 1.0 gm. per kg. body weight by stomach tube). A portion of the femoral or external jugular vein is exposed and a loose ligature is adjusted to facilitate injection into the vein. The carotid artery on the side of the sensitized eye is similarly prepared. A syringe with a very small hypodermic needle is used for the injections.

Epinephrine in saline solution is injected intravenously at intervals of about 5 minutes. When it is observed that the reacting structures are becoming more sensitive the injections are made directly into the carotid artery on the side of the sensitized eye. The injections are repeated, as in the case of the intravenous injections, until sensitivity of the reacting structures is markedly increased. When this occurs the animal can be used for assay of an unknown which contains an epinephrine concentration within the range of sensitivity of the test object.

Quantities ranging from 0.1 cc. to 1.0 cc. of the epinephrine solutions are used, usually beginning with 0.25 cc., 0.5 cc. and 1.0 cc.

in the intravenous injections. It is rarely necessary to use more than 0.5 cc. quantities in the carotid artery injections. Concentrations of epinephrine should be such as to yield small but unequivocal reactions. In the beginning it usually requires about 0.5 cc. of 1:1,000,000 to 1:2,000,000 to cause a distinct reaction when injected intravenously. At this stage reactions of the same intensity are produced by solutions of epinephrine 10 to 20 times as dilute, if injected into the carotid artery. A reaction obtained by injecting a given amount of epinephrine solution in a certain concentration is obtained with half that amount if the concentration is double or with double the amount if the concentration is half the original.

A much greater increase in sensitivity is ultimately produced by direct injections into the carotid artery than by the intravenous injections. With injections of 0.2 cc. to 0.5 cc. quantities, epinephrine concentrations of 1:100,000,000 to 1:200,000,000 commonly can be detected with certainty; sometimes concentrations of 1:300,000,000 to 1:500,000,000 have been detected and in one case definite reactions were obtained with solutions containing 1:1,600,000,000 epinephrine. The maximum sensitivity lasts for a while and then gradually diminishes. Quantitative determinations should be made as soon as the increase is large enough. They can be repeated if further increase in sensitivity occurs.

Usually the iris is the most sensitive of the reacting structures. Sometimes, however, the nictitating membrane reacts more readily and the widening of the palpebral aperture is also more definite than the pupil dilatation. This is often the case when the pupil is already considerably dilated before the epinephrine is injected. In a good reaction all of the structures participate. It should be remembered that substances other than epinephrine might occur in some blood specimens which may cause dilatation of the sensitized pupil. In this respect the intestine and uterus method is more reliable than the method under discussion. We have found, in one instance, that systemic blood-serum obtained from an animal in poor condition and under asphyxia caused a small pupil reaction which, however, was not difficult to distinguish from a larger epinephrine reaction.

The reaction to epinephrine in serum is the same as when the dilutions are made in saline solution. In case of intra-arterial injections the reactions occur in about 2 to 4 seconds; with intravenous injection they appear in about 6 to 15 seconds, according to the condition of the circulation in the animal. It is important that the rate of injection be constant in all observations. Especially in case of the intra-arterial injections, is it necessary to avoid sudden in-

jection since the sudden increase in pressure may result in a spurious dilatation of the pupil. On the other hand, if the injection is too slow the reaction may fail, particularly when working with high dilutions of epinephrine. It should be aimed to introduce the material at about the same rate as that of the blood flow in the blood vessel. A mechanical device has been found useful for this purpose.

We have compared the results of assay of epinephrine in serum of adrenal vein blood and of systemic blood to which epinephrine was added, using this method and the rabbit intestine method as employed by Stewart and Rogoff. The results obtained by the two methods compared remarkably well in practically every instance.

Since it is known that the epinephrine in blood is contained in the plasma or serum,[5] it is possible, by using serum for the tests, to study problems related to hyperepinephremia alleged to exist in various diseases. Any concentration of epinephrine found in the serum would correspond to about half that concentration in the whole blood. Thus, the test object, when sensitized to the degree found possible in the present investigation, could detect epinephrine in the systemic circulation if hyperepinephremia exists. Studies on the relation of the adrenals to hypertension and on the existence of detectable quantities of epinephrine in the circulation are in progress.

9264 P

Use of Air in Basal Metabolism.

C. V. PERRILL AND K. K. JONES. (Introduced by A. C. Ivy.)

From the Departments of Physiological Chemistry and Physiology, Northwestern University Medical School.

In the past there has been no reference in the literature to the use of air instead of oxygen in the semi-portable type of basal metabolimeters which are commonly used clinically. Yet, obviously, there may be certain advantages to using air instead of oxygen in a clinical metabolimeter. An article describing an air-using machine has just been published in a South American periodical by a doctor in Mexico City.[1] In certain parts of the world, oxygen is expensive and difficult to obtain.

It has been definitely shown[2] that the oxygen tension of the air

[5] Stewart, G. N., and Rogoff, J. M., *J. Pharm. Exp. Therap.*, 1917, **9**, 393.

[1] Macías, F. I., *Semana méd.*, 1936, **1**, 680.

[2] "Medical Studies on Aviation," *J. Am. Med. Assn.*, 1918, **71**, 17, 1383.

breathed can fall decidly before any detrimental physiological changes are noted, and Schneider[3] and others have found that the oxygen intake begins to fall only when the oxygen concentration in the air breathed is 14.8% or lower.

Our first experiments using air were carried out on a standard basal metabolimeter* which uses a motor-driven fan for circulating the gas in the closed system. Air was merely substituted for oxygen, and the tests were limited to periods of 4 minutes, as the total capacity of the machine was only 12 liters. Oxygen control

TABLE I.
Four-minute periods.

Subject	Date	Minute Volume		Deviation
		Oxygen cc.	Air cc.	%
L. N.	1-20	256	262	2.3
J. S.	1-23	213	208	−1.9
R. K.	1-26	280	278	−0.7
G. L.	2-13	276	276	0.0
	Totals	1025	1024	
		Average deviation		−0.1

TABLE II.
Preliminary Series. Six-minute periods.

Subject	Date	Minute Volume		Deviation	Gas Analysis
		Oxygen cc.	Air cc.	%	%
W. C.	7-11	233	223	−4.3	16.7
,,	11	233	230	−1.3	
K. J	15	297	297	0.0	16.0
,,	16	265	275	3.7	14.8
,,	19	257	252	−1.9	14.4
,,	20	294	296	0.9	15.3
,,	20	289	278	−3.9	
C. P.	20	270	273	1.2	
,,	27	284	271	−4.7	14.1
K. J.	27	318	322	1.4	
C. P.	28	270	275	1.6	
,,	29	276	267	−3.0	15.0
,,	30	291	285	−2.3	
,,	30	293	288	−1.8	
,,	8-3	298	302	1.2	14.1
,,	3	303	278	−8.1	
,,	6	289	271	−6.1	14.2
,,	6	267	250	−6.3	
W. C.	9-29	218	228	4.6	
Totals		5255	5161		
		Average deviation		−1.8	

[3] Schneider, E. C., Truesdell, D., Clarke, R. W., *Am. J. Physiol.*, 1924, **70**, 283.

* "Sanborn Motor-Grafic, Model E-I-S." Manufactured by the Sanborn Company, Cambridge, Mass.

tests were made immediately preceding and following each test using air. Four different subjects were used. It was found that the results of the tests in which air was used agreed to 0.1% with those in which oxygen was used.

As a 4-minute test was not considered to be a period of sufficient length for a good determination, an auxiliary tank of air was connected in series with the metabolimeter. Using a 6-minute

TABLE III.
New Machine. Six- and 8-minute periods.

Subject	Date	Minute Volume		Deviation
		Oxygen cc.	Air cc.	%
B. L.	11-2	225	238	5.7
H. D.	4	190	197	3.4
S. K.	6	202	182	−9.9
F. B.	7	162	157	−3.1
H. P.	8	233	221	−5.1
”	8	233	230	−1.6
C. P.	12	266	259	−3.0
”	13	272	265	−1.8
	Totals	1783	1749	
		Average deviation		−1.9

period, a preliminary group of 20 trials and 19 control determinations were made with this apparatus on 3 normal subjects. The average of the results of these 6-minute periods agreed within 1.5% with the results obtained with oxygen. (Table II.)

These results caused us to construct a machine with the spirometer incorporated into an auxiliary tank. The construction of this machine is shown in the diagram. The oxygen consumption is measured by the fall of the spirometer which is recorded on a kymograph drum in the usual way. The spirometer was calibrated by carefully measuring the distance traversed by the pen on the kymograph drum when air was added or subtracted in 500 cc. increments from the machine. The results on patients were also compared and found to agree with data obtained with a Benedict machine. Our machine was constructed from plans calculated to give it a total capacity of 40 liters, which was sufficient to provide the patient with fresh air for the entire 6-minute period of the test. Circulation of the air within the apparatus was maintained by means of a small 6-volt motor, driven by a storage battery. This motor did not heat the air to any measurable extent.

It was noticed that a rise of temperature of one degree centigrade produced an error of about 4% in this machine. In order to avoid

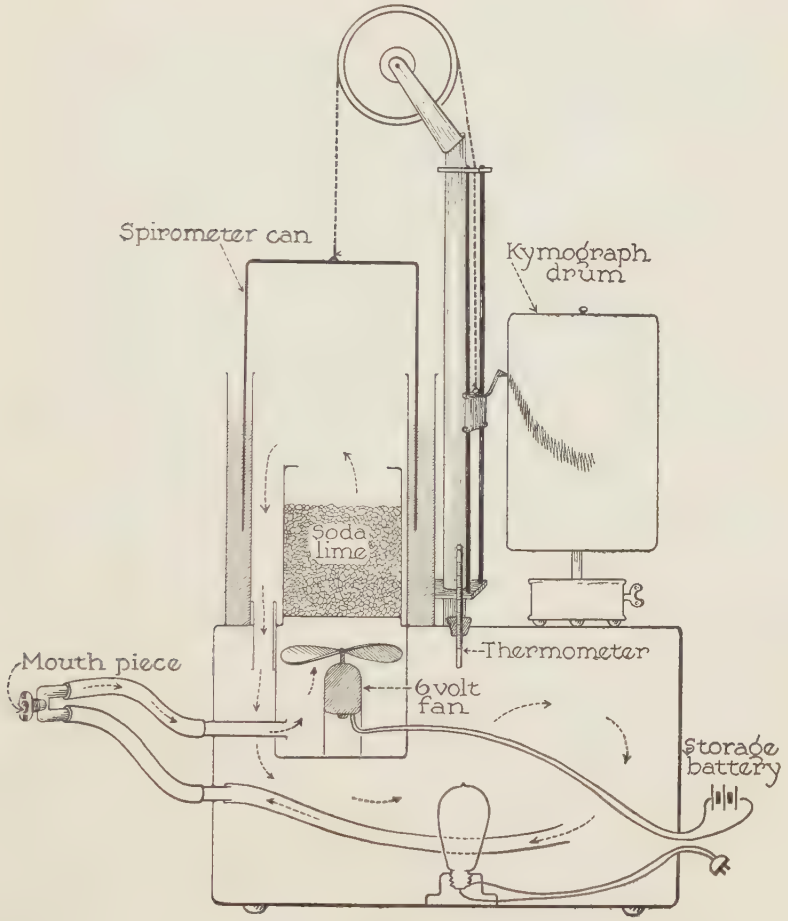

FIG. 1.
Scale diagram of metabolism machine constructed to use air instead of oxygen. Capacity of tank and spirometer = 40 liters.

this source of error, the air was heated before each test by a light-bulb inside the machine. The temperature during the test was then found to remain constant.

Checks on the oxygen level in the various containers were obtained by means of gas analyses, using a modified form of the Henderson-Orsat gas analysis machine.[4] In the preliminary series of tests, the oxygen concentration fell from 20.9%, to between 14 and 15%. (Table II.) In the tests with the 40 liter machine it fell to 16%, which is not below the oxygen concentration in ordinary expired air,[5] and indicates a wide margin of safety for this machine.

[4] Henderson, Y., *J. Biol. Chem.*, 1918, **33**, 31.

[5] Du Bois, E. F., "Basal Metabolism in Health and Disease," Lea & Febiger, 1924, p. 64.

An average of 8 determinations of metabolism with this machine on 6 different subjects gave results which varied by less than 2% from that obtained under exactly similar conditions with a standard machine. This is within the requirements of clinical calorimetry.

9265 P

Total Thyroidectomy for Human Diabetes Insipidus.

THOMAS FINDLEY, JR., AND PETER HEINBECKER.

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Among the indications that the thyroid may play an important rôle in water metabolism are several pieces of evidence which suggest that total thyroidectomy might appreciably ameliorate the symptoms of human diabetes insipidus: Mahoney and Sheehan[1] abolished experimental temporary diabetes insipidus in dogs by this procedure and reëstablished the polyuria by feeding thyroid substance; Fisher and Ingram[2] obtained similar though less striking results in cats; others[3, 4] found that in thyroidectomized dogs anterior pituitary extract had no diuretic effect; and Strauss[5] once saw clinical diabetes insipidus disappear with the onset of spontaneous myxedema. It was anticipated that species differences might spell failure in the human subject, for anterior lobe extracts do not induce polyuria in normal rats[6] and Stern and Gilligan[7] found that the responses to water-drinking and to pituitrin are similar in normal subjects and in those with artificial myxedema. Our patient was fully aware of the experimental nature of the proposed operation and willingly cooperated. We have seen no previous reports of similar studies in man or monkeys.

W. D., a 55-year-old colored male with central nervous system syphilis and diabetes insipidus of 3 years' duration which had not yielded to antiluetic therapy, underwent complete thyroidectomy on

[1] Mahoney, W., and Sheehan, D., *Am. J. Physiol.*, 1935, **112**, 250.

[2] Fisher, C., and Ingram, W. R., *Arch. Int. Med.*, 1936, **58**, 117.

[3] Barnes, B. O., Regan, J. F., and Bueno, J. G., *Am. J. Physiol.*, 1933, **105**, 559.

[4] Biasotti, A., *Compt. rend. Soc. de biol.*, 1934, **115**, 329.

[5] Strauss, L., *Deutsche med. Wchnschr.*, 1920, **6**, 939.

[6] White, H. L., *Am. J. Physiol.*, 1937, **119**, 5.

[7] Stern, B., and Gilligan, D. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 843.

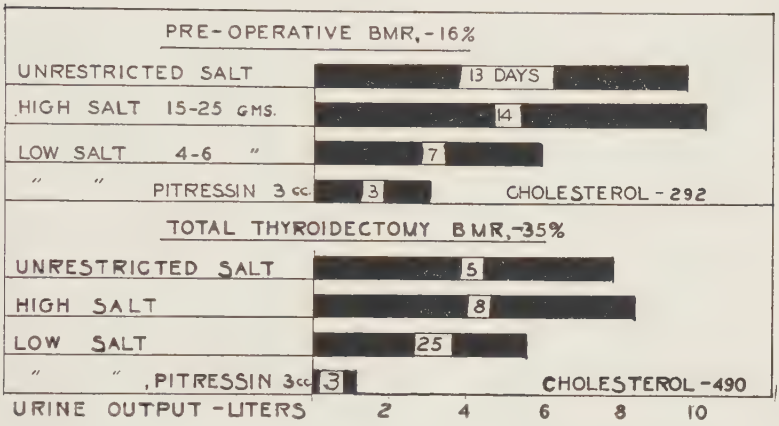

FIG. 1.

Urine Output Before and After Total Thyroidectomy.

Each rectangle represents the average 24-hour urine flow for the number of days enclosed within it. Fluid intake was unrestricted throughout; the values are not recorded because they closely paralleled the urine volumes in every period.

August 1, 1936. The results will be discussed in detail elsewhere but the accompanying chart shows that no striking diminution in urine output resulted. On a low-salt diet the average 24-hour output of urine was essentially unchanged by thyroidectomy, but urine volume was increased less by salt administration and diminished more by pitressin after operation than before. It is difficult to evaluate subjective reactions but it seems worth recording that the patient insists that the thirst and polyuria are much less distressing than before. Our conclusion, however, is that total ablation of the thyroid is about as effectively antidiuretic as a low-salt diet.

Conclusions. Total thyroidectomy on a case of clinical diabetes insipidus diminished the diuretic effect of sodium chloride and increased the antidiuretic effect of pitressin. It failed, however, to reduce urine flow below the limits set by a low-salt diet.

9266

Diminished Effectiveness of a Second Administration of Atropine or Novatropin. Mechanism of Recovery.

J. P. Quigley.

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.

Administration of atropine preparations over a period of time leads in certain animals to a decrease in the effectiveness of the drug. Cloetta[1] found that normal rabbits excrete a dose of atropine during a period of 2 or 3 days but excretion is complete in one day according to Fickewirth and Heffter[2] if atropine is administered to rabbits habituated to the drug. Normal cats excrete very little atropine (Doeblin and Fleischmann[3]) but after habituation, the excretion is markedly augmented.

Another type of increased resistance to atropine preparations was noted in a series of experiments in this laboratory performed on fasting dogs and man in which objective records of gastro-intestinal motility were made by the balloon method. (The triple, tandem balloon was used in the human experiments.) It was thus determined that injection of an atropine preparation shortly after recovery (*i. e.*, return to preinjection tone and motility) from a preceding administration of atropine was less effective than the initial injection.

When administered as the initial medication, 0.065 mg./Kg. atropine intravenously administered to the unanesthetized dog uniformly completely inhibited the stomach and recovery was complete in 80 ± 30 minutes. (Results of initial medications are presented in detail elsewhere.) Following complete return of preinjection tone and motility, a second injection of the same quantity of atropine (8 experiments) produced inhibition with recovery in approximately 30 minutes (3 experiments) or failed to produce complete inhibition (5 experiments). Reduced effectiveness from a second administration was likewise demonstrated when both the first and second dose were smaller (0.032 mg./Kg.) or larger (0.13 mg./Kg.). For example, the initial administration of 0.13 mg./Kg. produced complete gastric inhibition with recovery in 150 ± 30 minutes. Repetition of this dosage after recovery (4 experiments) produced transient complete inhibition (3 experiments) or incomplete inhibition (1 experiment).

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[1] Cloetta, *Arch. exp. Path. Pharm.*, 1911, **64**, 427.

[2] Fickewirth and Heffter, *Biochem. Z.*, 1912, **40**, 36.

[3] Doeblin and Fleischmann, *Z. klin. Med.*, 1913, **77**, 145.

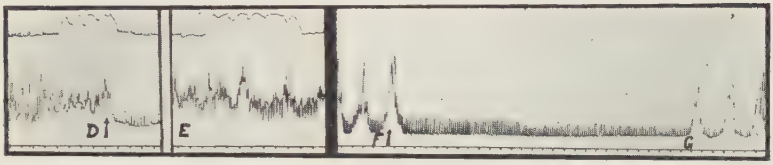

FIG. 1.

Effect of Novatropin and atropine on gastric motility.

Upper line, colon; mid-line, stomach; lower line, time intervals in minutes.

D. effect of intravenous injection of 0.065 mg./Kg., atropine sulphate, initial medication; E. early stage of complete recovery of stomach 62 minutes after injection.

F. following apparently complete recovery from a previous injection of atropine, injected intravenously 0.065 mg./Kg. atropine sulphate; G. early stage of complete recovery 29 minutes after injection.

Gastric hypermotility is produced by administration of insulin (Quigley, Johnson, Solomon[4]) This hypermotility in the normal human was uniformly completely inhibited by 0.65 mg. atropine administered subcutaneously when given as the initial medication and recovery was complete in 45 ± 15 minutes. However, when given as the second injection (8 experiments), this dose either produced only a transient complete inhibition (5 experiments), or inhibition was incomplete (3 experiments).

Reduced effectiveness from repetition of administration was also observed with Novatropin (methylhomatropine bromide), a drug with an atropinelike action. Initially, 0.1 mg./Kg. in the dog always produced complete gastric inhibition with complete recovery in 55 ± 10 minutes. After recovery, a second injection of this drug in 6 experiments produced no effect in one experiment, incomplete inhibition with complete recovery in 25 minutes in 3 experiments and in 2 experiments complete inhibition developed but was followed by full recovery in 45 minutes. As the initial medication, 0.2 mg./Kg. Novatropin in the dog produced complete gastric inhibition with full recovery in 90 ± 25 minutes (3 experiments). A repetition of this dose failed to produce complete inhibition in 2 experiments and in one experiment inhibition was complete but full recovery occurred in 25 minutes. Similar results with Novatropin were obtained in man.

A decrease in effectiveness was obtained in the dog or human when atropine followed Novatropin or *vice versa*. This is illustrated by 3 experiments on man, in each of which 1.3 mg. atropine was given after recovery from 1.5 mg. Novatropin. In one experiment, complete inhibition developed but recovery was complete in 39 minutes. In another case, the region of the upper 2 balloons was

[4] Quigley, Johnson, Solomon, *Am. J. Physiol.*, 1929, **90**, 89.

completely inhibited for 31 minutes but the region of the lower balloon was unaltered. In the third experiment, the only effect consisted in a partial inhibition lasting for 3 minutes.

In 8 experiments with atropine and 6 experiments with Novatropin the second injection was found to inhibit motility of the colon in dogs as effectively as the initial injection of these drugs.

Although tolerance against the action of atropine preparations following chronic administration of atropine preparations is due to the development of an increased rate of elimination, this mechanism rather definitely does not explain the reduced effectiveness of a second injection as noted in our experiments. The return of preinjection gastric tone and motility is not an indication of complete disappearance of atropine or Novatropin from the organism but apparently occurs when the concentration is only moderately reduced. A further elevation of atropine or Novatropin concentration (probably to a level above the previous maximum) by administration of a second dose of these drugs may produce a partial inhibition of the stomach but the depression is usually only moderate because the tissue has developed a resistance to the action of these drugs; *i. e.,* the motility obtaining at the time of recovery exists in spite of the presence of the drug. This observation would have the important clinical indication that little effect on gastric motility or tone should be anticipated from a second administration of atropine preparations after recovery from a previous administration of this drug.

The colon probably also recovers motility in spite of the continued presence of atropine or Novatropin but at the time of recovery from the initial injection is less resistant to a further augmentation of the drug than is the stomach.

9267 P

Chronic Uterine Distention in Oestrin-Treated Rabbits.*†

Samuel R. M. Reynolds.

From the Department of Physiology, Long Island College of Medicine, Brooklyn.

When the uterus of an untreated, ovariectomized rabbit is distended for 2 weeks with a paraffin pellet of suitable size a certain degree of growth takes place.[1] The amount of growth depends in part upon the amount of stretching to which the uterus is subjected. As shown by the curve, the pellet must be more than one-half and less than twice the size (cross-sectional area) of the undistended uterus (including the lumen)[1] to be an effective stimulus. The present experiments were carried out to learn the effect of chronic uterine distention *in uteri* of ovariectomized rabbits which were first enlarged under the influence of 800 r.u. of oestradiol over a period of 2 weeks. The pellets were left *in utero* for 2 weeks and an additional 400 r.u. of oestradiol was given. The tissues were obtained and the degree of growth ascertained in the manner previously described.[1]

In the present work, the range of distention was at one extreme a uterus:pellet ratio of 3½ and at the other, a uterus:pellet ratio of somewhat less than one-half (Fig. 1). Twenty-six distention sites from 15 different rabbits were obtained. The distribution of these points is shown in Fig. 1, curve A, along with the percentage increase or decrease of the distended portions of the uterus relative to the undistended parts. Curve B is the form of the growth curve obtained in untreated, ovariectomized rabbits.[1]

The present results show, therefore, that the effect of oestrin acting upon a chronically distended uterus is to reduce markedly the capacity of the uterus to grow in response to the stimulus of distention. At the left of curve A, it will be seen that the distention sites are smaller than the undistended parts of the same uterus. Clearly, therefore, a summation of the growth responses resulting from distention and the growth resulting from the action of oestrin is not found in these experiments. Furthermore, it is found that

* Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

† A full report of these findings will be given in a paper at The Biological Laboratory, Cold Spring Harbor, during the summer of 1937, as part of a Symposium on Internal Secretions. Publication will be made with the Proceedings of that Symposium.

1 Reynolds, S. R. M., and Kaminester, S., *Am. J. Physiol.*, 1936, **116**, 510.

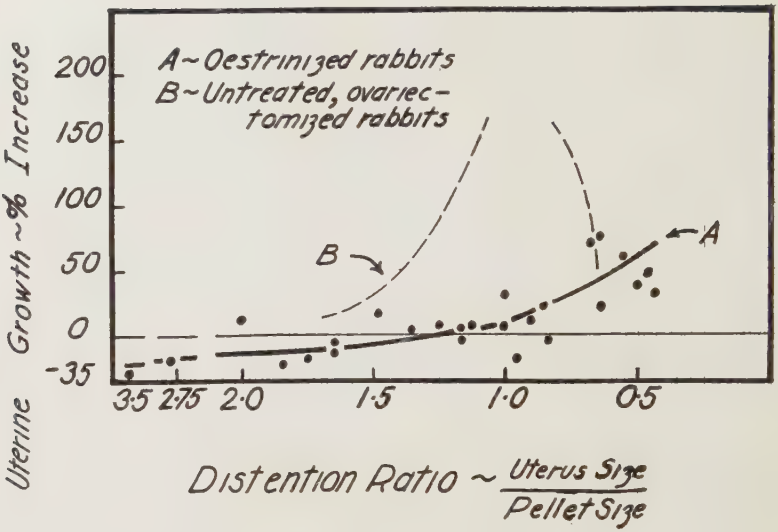

FIG. 1.

throughout nearly the whole range of distentions in the present experiments, the endometrium at the distention sites is smaller than is the endometrium at the undistended sites, while the myometrium usually shows some growth, especially with the larger degrees of distention. Even so, the growth is very much less than that observed in the myometrium of untreated, ovariectomized rabbits with comparable degrees of distention.

These effects are best explained on the basis of impoverishment of the blood supply to distended parts of the uterus owing to the increased tonicity of the myometrium under the influence of oestrin associated with the presence of the pellet in the uterus. The physiological significance of these results in relation to gestation in the rabbit is discussed elsewhere.[2]

Summary. Chronic uterine distention in oestrin-treated rabbits results in a reduction of the capacity of the uterus to grow in response to the stimulus of distention.

[2] Reynolds, S. R. M., *Am. J. Obstet. Gynecol.*, 1937, **33**.

9268 P

Chronic Uterine Distention in Progestin-Treated Rabbits.*†

SAMUEL R. M. REYNOLDS AND WILLARD M. ALLEN.

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y., and the Department of Obstetrics and Gynecology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

The present report deals with the effect of progestin on the distention-growth response of the uterus as described before.[1] Paraffin pellets were inserted into the uterus *per vaginam* in rabbits ovariectomized for one week. At the same time, 200 r.u. of oestradiol were given subcutaneously. Commencing two days later, 6.6 rb.u. of progestin was given in the next 10 days. On the thirteenth day of uterine distention, tissues were taken, prepared and studied in the manner described before.[1] The dosage of progestin was about liminal for maintenance of endometrial proliferation for a period of 10 days.[2] Two groups of rabbits were used. One consisted of 7 mature rabbits from which 13 different distention sites were available; the other group consisted of 9 immature rabbits from which 14 distention sites were available. The uterus:pellet ratio was 2.89 at one extreme and 0.26 at the other. In mature rabbits, growth resulting from distention was regularly obtained. Curve A, Fig. 1, shows the form of the curve in these rabbits, in contrast to that obtained in untreated rabbits, curve B. Here it is seen that the optimum uterus:pellet distention ratio for growth is approximately 1:2 for curve A, whereas that for curve B is 1:1. The extremes of the 2 curves, insofar as they go, bear a similar relationship to each other. Clearly, therefore, increased stretching of the tissues is essential in these rabbits to elicit an amount of growth comparable to that produced by less distention in untreated, ovariectomized rabbits. Thus, progestin serves to raise the threshold for the growth response to chronic uterine distention of the uterus. Although both the endometrium and myometrium contribute to the enlargement which takes place, the myometrium grows perceptibly more than the endometrium.

* Aided by a grant from the Committee for Research in Problems of Sex, of the National Research Council.

† A full report of these findings will be given as part of a paper in the Symposium on Internal Secretions at the Biological Laboratory, Cold Spring Harbor, during the summer of 1937. Publication will be made in the Proceedings of that Symposium.

1 Reynolds, S. R. M., and Kaminester, S., *Am. J. Physiol.*, 1936, **116**, 510.

2 Allen, W. M., and Heckel, G. P., *Anat. Rec.*, 1936, **64**, 2.

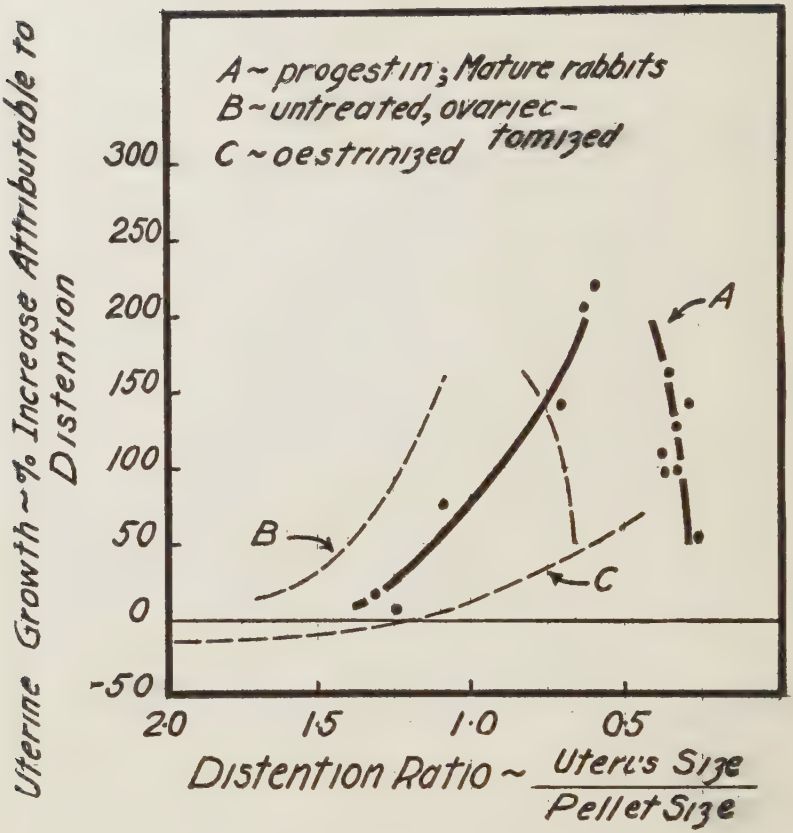

FIG. 1.

In immature rabbits treated with progestin, little or no growth resulting from distention is produced. All points in this series fall along curve C, Fig. 1, and so are classed with the data obtained in oestrin-treated rabbits. All the uteri of these progestin-treated rabbits showed good endometrial proliferation, with the possible exception of the 2 distention sites showing the greatest degree of uterine enlargement (curve A). These tissues were less proliferated than were those with smaller amounts of growth.

Summary. In mature, progestin-treated rabbits, growth resulting from distention is regularly obtained, but greater degrees of distention are necessary to produce growth responses comparable to that obtained in untreated, ovariectomized rabbits with smaller amounts of stretching. It is probable that progestin, through its property of reducing myometrial tonicity, may reduce the tension of the tissues about the pellets to such an extent that greater degrees

of stretching are required in order to produce a degree of tension equal to that obtained by less distention in untreated, ovariectomized rabbits. Hence, an appropriate amount of tension appears to be an essential condition for uterine enlargement to take place as a result of distention. In oestrin-treated rabbits, however, the distention-growth response is much reduced.[3] This has been attributed to impairment of the blood supply about the sites of distention, resulting from the great increase in tonicity of the myometrium due to oestrin. Thus distention appears to be an adequate stimulus for uterine growth when a small degree of tension is produced, but not if the tension is so great as to interfere with the nutrition (blood supply) of the tissues.

9269

Failure to Obtain in Immature Rabbits Uterine Growth by Chronic Distention.*

SAMUEL R. M. REYNOLDS AND SANFORD KAMINESTER.†

From the Department of Physiology, Long Island College of Medicine, Brooklyn.

In the course of studying the local growth which takes place in chronically distended uteri in both untreated, ovariectomized rabbits and in progestin-treated rabbits,[1-4] 12 immature rabbits were employed. In the untreated ovariectomized rabbits, 6 separate distention sites were studied, and compared with undistended areas of the same uteri. Lack of sexual maturity was evidenced by the presence of infantile uteri and small, flat ovaries without macroscopic Graafian follicles. It was found that growth resulting from distention was not obtained in any instance, even though the degrees of distention were similar to those which yielded large growth responses in mature rabbits.[2, 4]

In the 9 progestin-treated immature rabbits, 14 distention sites

3 Reynolds, S. R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 453.

* Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

† Member of the Department of Obstetrics and Gynecology.

1 Reynolds, S. R. M., and Kaminester, S., *Am. J. Physiol.*, 1936, **116**, 510.

2 Reynolds, S. R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 453.

3 Reynolds, S. R. M., and Allen, W. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 455.

4 Unpublished data on the rate of the distention-growth response.

were available. These rabbits received 200 r.u. of oestradiol in oil subcutaneously, and a total of 6.6 rb.u. of progestin in 10 days commencing the third day after the oestradiol was given. Despite the fact that the endometrium in each of these rabbits was well proliferated (proving that the oestrin and progestin were effective) the distention sites failed to show appreciable enlargement as compared with the undistended portions of uterus. The degrees of distention were comparable to those which yielded growth responses at 13 distention sites in a total of 7 mature progestin-treated rabbits.[1]

These results show that local uterine growth, which takes place with suitable degrees of chronic distention in sexually mature rabbits, depends upon some factor or conditions associated with maturity. It is not dependent, however, upon influences of the known ovarian hormones since oestrin and progestin administered to immature rabbits were effective, causing endometrial proliferation without inducing the distention-growth response.

9270

Action of Synthetic Vitamin B_1.

CHARLES R. ECKLER AND K. K. CHEN.

From the Lilly Research Laboratories, Indianapolis, Indiana.

The antineuritic vitamin, B_1, has been crystallized by different groups of investigators,[1-6] but it remained for Williams and his associates[7] to arrive at a correct structural formula, confirmed by successful synthesis.[8]

For some time, the crystalline vitamin B_1, both the natural and the synthetic, has been available in the form of the hydrochloride in our

[1] Jansen, B. C. P., and Donath, W. F., *Geneesk. tijdschr. v. Nederl. Indie*, 1927, **66**, 810.

[2] Odake, S., *Proc. Imp. Acad. Tokyo*, 1931, **7**, 102.

[3] Windaus, A., Tschesche, R., Ruhkoff, H., Laquer, F., and Schultz, F., *Z. f. physiol. Chem.*, 1932, **204**, 123.

[4] Seidell, A., and Smith, M. I., *J. Am. Chem. Soc.*, 1933, **55**, 3380.

[5] Kinnersley, H. W., O'Brien, J. R., and Peters, R. A., *Biochem. J.*, 1933, **27**, 232.

[6] Williams, R. R., Waterman, R. E., and Keresztesy, J. C., *J. Am. Chem. Soc.*, 1934, **56**, 1187.

[7] Williams, R. R., *J. Am. Chem. Soc.*, 1935, **57**, 229; 1936, **58**, 1063.

[8] Williams, R. R., and Cline, J. K., *J. Am. Chem. Soc.*, 1936, **58**, 1504; 1937, **59**, 216.

laboratory for pharmacological studies. Our results appear so uniform that they can probably serve as an additional proof to that established by chemical means, concerning the identity of the two products.

To determine the potency of the synthetic vitamin B_1, rats were made definitely polyneuritic and treated with various doses according to the technique of Smith,[9] except that a small amount (0.4%) of Brewers' yeast was added to the diet, as suggested by Dann,[10] in order to reduce the incidence of deaths. Tests were also carried out in pigeons rendered polyneuritic by the diet proposed by Cowgill[11] for confirmative purposes.

TABLE I.
Potency of Synthetic Vitamin B_1.

Species Used	No. of Polyneuritic Animals Used	Dose mg.	No. of Animals Cured
Rats	3	.003	1
	7	.004	6
	13	.005	10
	5	.006	4
Pigeons	9	.005	9

As shown in Table I, doses of 0.004 to 0.006 mg. of synthetic vitamin given by mouth were sufficient to cure 77 to 85% of the rats. An amount of 0.003 mg. was effective in one case but failed in the other 2, so that the minimal curative dose of the vitamin by mouth in rats was 0.004 mg. in the present series of experiments. In 9 pigeons, a dose of 0.005 mg. injected intravenously abolished the symptoms of polyneuritis in 4 animals for 2 to 3 days, and in 5 for 4 days or more. The figures in this investigation with both rats and pigeons correspond closely to those reported by other workers for the natural crystalline vitamin,[6, 12-14] and fully confirm those claimed by Williams and Cline[8] for their synthetic product.

Other pharmacological effects are also similar when the natural and the synthetic compounds are compared side by side. In an etherized cat weighing 2.63 kg., doses of 5, 20, and 50 mg. of the synthetic product, injected intravenously, caused a slight decrease

9 Smith, M. I., *Pub. Health Rep.*, 1930, **45**, 116.

10 Dann, F. P., *J. Nutrition*, 1936, **12**, 461.

11 Cowgill, G. R., The Vitamin B Requirement of Man, Yale Press, New Haven, 1934, 29.

12 Jansen, B. C. P., Kinnersley, H. W., Peters, R. A., and Reader, V., *Biochem. J.*, 1930, **24**, 1824.

13 Ammerman, M., and Waterman, R. E., *J. Nutrition*, 1935, **10**, 25.

14 Waterman, R. E., and Ammerman, M., *J. Nutrition*, 1935, **10**, 161.

in respiratory volume, but no changes in respiratory rate, blood pressure, or heart rate. Similar effects were observed with the same doses of the natural vitamin. Neither substance produced congestion or necrosis in the rabbit's ear by subcutaneous injection in the dosage of 1 mg. dissolved in 0.1 cc. of saline. The minimal lethal dose (M.L.D.) in guinea pigs is the same with both the natural and the synthetic products, by intravenous injection, as shown in Table II. The vitamin solution employed for the toxicological study was 2% in each case, and the weight of the animals varied from 210 to 265 gm. Clonic convulsions occurred after doses of 150 mg. per kg., or more, had been administered. Those animals which survived the sublethal doses recovered completely within 1½ and 5 minutes, apparently without any after effects.

TABLE II.
Toxicity of Natural and Synthetic Vitamin B_1 Hydrochloride.

Vitamin B_1.HCl	Dose mg. per kg.	No. of Pigs Died Over No. Used	M. L. D. mg. per kg.
Synthetic	300	1/1	180
	200	1/1	
	180	2/3	
	160	0/2	
	150	0/1	
	100	0/1	
Natural	180	2/2	180
	160	1/3	

Summary. Results obtained in animals indicate that the natural crystalline vitamin B_1 and the synthetic product are identical.

9271 P

Increased Estrogenic Potency of Human Urine after Hydrogenation.

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A method described by us[1] for the hydrolysis and extraction of urine for estrogens involves boiling with 15 vol. % of HCl for 10

[1] Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.

minutes, followed by continuous extraction with benzene (Benzol, Merck's Reagent) for 24 hours. It has been shown that the benzene extraction recovers all estrogens present after hydrolysis. It has also been demonstrated that this short hydrolysis with a high concentration of acid gives values which check with those obtained after the Cohen and Marrian[2] technique,* and does not affect the potency of estrone or estriol added to urine of known "total" estrogenic content.

Although the application of this method of quantitation has been found to yield consistent physiological curves of estrogen excretion,[3, 4] there is no proof that all bound estrogens are freed by the acid hydrolysis or that destruction of combined estrogens may not occur. With the idea of possibly increasing hydrolysis as well as preventing any destruction of combined estrogens through oxidation,

TABLE I.

Comparative Estrogenic Potency of Human Urines Hydrolyzed With and Without Addition of Zinc.

Name	Date	Clinical Notes	Total Estrin T_o rat units–24 hr.	Total Estrin T_{zn} rat units–24 hr.	T_{zn}/T_o
Y.B.	8/ 1/36	5 mo. pregnant	*12000	*28000	2.33
,,	9/23/36	6½ ,, ,,	*28000	*75000	2.67
,,	11/26/36	8½ ,, ,,	*64000	*130000	2.05
L.	1/30/37	3 ,, ,,	1800	7250	4.0
		Results on Specimens Collected Throughout a Menstrual Cycle.			
		Menstruating days of cycle			
H.S.	1/12-14	1-3	20	134	6.6
	1/14-16	3-5	33	134	4.0
	1/16-18	5-7	55	110	2.0
	1/18-20	7-9	100	220	2.2
	1/20-22	9-11	270	890	3.3
	1/22-24	11-13	200	670	3.3
	1/24-26	13-15	100	333	3.3
	1/26-28	15-17	100	333	3.3
	1/28-30	17-19	134	450	3.3
	1/30-2/1	19-21	134	450	3.3
	2/1-3	21-23	134	450	3.3
	2/3-5	23-25	55	134	2.5
	2/5-7	1-3	33	220	6.6
	2/7-8	3-4	20	134	6.6

* Assays made on unextracted specimens diluted with water. All other specimens were extracted with benzene and assayed in olive oil solution.

[2] Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1934, **28**, 603.

* The Cohen and Marrian hydrolyses were performed in the Biological Laboratories of Harvard University through the courtesy of Dr. Gregory Pincus. Assays, by the Allen-Doisy method, were made in this laboratory.

[3] Smith, G. V., and Smith, O. W., *New Eng. J. Med.*, 1936, **215**, 908.

[4] Smith, G. V., and Smith, O. W., *Am. J. Obstet. and Gynec.*, 1937, **33**, 365.

zinc (Zinc Dust, Merck's Reagent) has been added to urines prior to the acid treatment. A marked rise in the estrogenic potency of urines from both pregnant and non-pregnant women has resulted (Table I).

"T_o" signifies the addition of 15 vol. % HCl and 10-minute boiling under a reflux condenser; T_{zn} the addition of 15 vol. % HCl and 4% Zn and 3-hour boiling under a reflux. Four percent zinc constitutes an excess with 15 vol. % HCl. Maximum increase in potency occurs after 2 hours and is not changed after 5 hours of boiling with acid and zinc. Evolution of hydrogen continues both during boiling and extraction. The titratable acidity is reduced from around 1.5 N to around 1.3 N in 3 hours of boiling. It is to be noted that hydrogenation does not affect a uniform increase in potency, since the ratios of T_{zn} to T_o, when assayed in olive oil, vary between 2.0 and 6.6.

The processes involved in this augmentation of urinary estrogen by zinc hydrolysis have not as yet been identified. The results thus far, however, are in accord with the hypothesis that the explanation lies in increased hydrolysis, and also conversion of estrone (but not estriol) into a reduced form of greater estrogenic activity, possibly dihydro-estrin (dihydro-theelin, estradiol). It is apparent for the present that hydrolysis with the addition of zinc may not be employed in physiological studies of estrogen excretion, although there is some indication that the ratio of T_{zn} to T_o may provide an index of the relative estrone content of specimens analyzed.

9272 P

Changes of Hydrogen Ion Concentration of the Cerebral Cortex.

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Using a glass electrode (of the MacInnes type) with an active area of less than 0.5 mm.^{2}, in conjunction with the microvoltmeter recently described by Burr, Lane and Nims (1936), having a grid-leak of 100 megohms, it is possible to measure the hydrogen ion concentration in physico-chemical systems to $\pm$.002 pH. The same apparatus is applicable to biological systems *in vivo*. In the present instance it was used for a study of the pH of the cerebral cortex.

One glass electrode and 2 Ag-AgCl saline-wick electrodes were placed as close together as possible (circa 2 mm.) on a selected area of the cerebral cortex of the animal. The potential difference between the wick electrodes was measured with a microvoltmeter and a Leeds and Northrup galvanometer (No. 2420). The E.m.f. between the glass electrode and either wick electrode was measured with the modified microvoltmeter and a similar galvanometer, in conjunction with a portable Leeds and Northrup potentiometer. The apparatus was so adjusted that the variations in these voltage differences could be recorded photographically with a moving-paper camera through its F/1.25 anastigmatic lens (focal distance 5 cm.). The 2 wick electrodes were placed on the cortex to determine whether or not potential gradients were so large or unstable as to invalidate a pH-measurement. The difference of potential between the glass electrode and either wick electrode can be correlated with a pH by standardization in buffers of known pH.

In this preliminary note we wish to confine ourselves to a few of the results thus far obtained.

1. The D.C. potential gradients are small enough to be neglected in estimating the pH to $\pm.05$, and stable enough throughout an experiment to permit differential measurements of pH to $\pm.005$, a precision more than sufficient for the measurements in question. 2. In the curarized animal (monkey, cat) under constant artificial respiration the indicated pH on the cortex is constant. Increase of ventilation produces a shift towards the alkaline side (see figure), decrease of ventilation one towards the acid side. In fact, it has been possible to maintain the pH on the cortex at any specified level compatible with life by proper adjustment of the ventilation. 3. Intravenous injection of sodium bicarbonate produces a shift towards the alkaline side, of hydrochloric acid towards the acid side, of Ringer-solution no comparable effect. 4. Thermocoagulation (at 80°C. for 5 seconds) of a small area of the cortex renders this area acid (*e. g.*, pH = 6.6) with respect to the adjacent normal cortex (*e. g.*, pH = 7.3). This acidity slowly increases during both the initial local vasoconstriction and the subsequent local vasodilatation and oedema of the thermocoagulated area.

From these findings we feel justified in concluding that with this method one measures pH, that, though the condition of the blood circulating through the cortex affects the indicated pH, this pH is that of the transudate on the surface of the cortex immediately subjacent to the glass electrode, and finally that the pH of this transudate is largely determined by the condition of that portion of the cortex, rather than merely reflecting its vascularity.

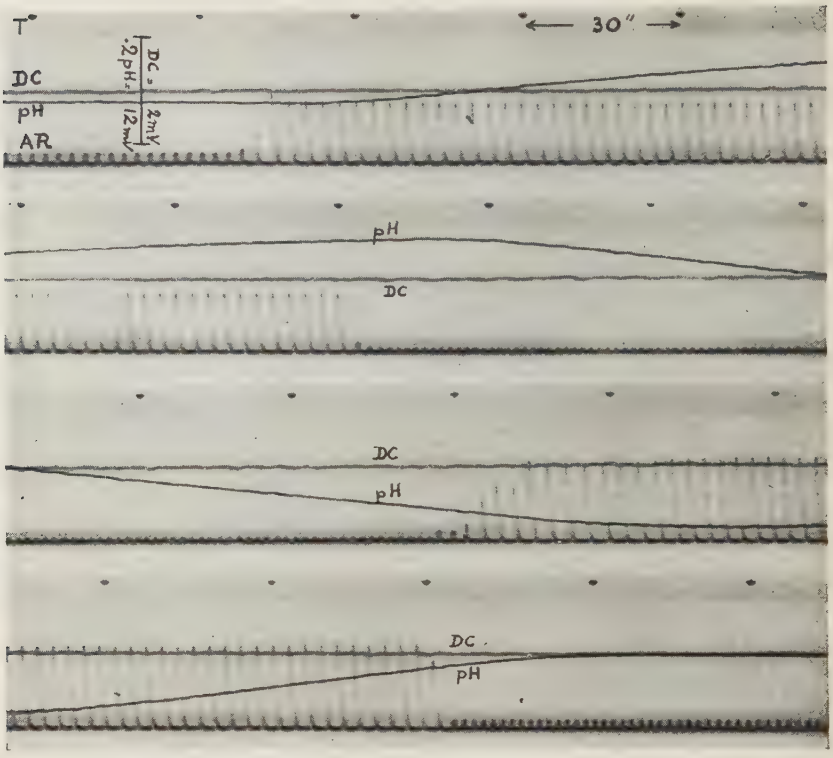

Fig. 1.

Monkey. Light Dial narcosis. Tracheal cannula. Curare. The four strips are continuous. T is time in half minutes. AR is artificial respiration. DC is potential between two wick electrodes about 2 mm. apart on cerebral cortex. pH is variation of potential between glass and one of the wick electrodes, also about 2 mm. apart on cortex; upward increase, downward decrease of pH.

Note that changes in artificial respiration are followed by shifts in pH without appreciable shift in D.C. potential, although the D.C. voltage sensitivity is 6 times as great.

5. Changes in pH of the cortex produce changes in its "spontaneous" electrical activity, a low pH being associated with low electrical activity, a high pH with high activity.

9273

Prostatic Type of Paraurethral Glands Induced in Female Rats by Administration of Male Sex Hormone.*

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On the basis of histologic[1] and embryologic[2] data the paraurethral glands (Skene's ducts) of women have long been considered homologous to the prostate of man, in particular to the cranial and ventral lobes.[3] In rats these glands have only recently been described[4] and studied embryologically.[5] It has later been reported that injection of the male sex hormone stimulates the growth and development of these glands in the female rat; in contrast they are not affected by oestrone.[6] In view of the scarcity of definite data on Skene's ducts and their relation to the formation of cysts[7] in women, it is deemed worthwhile to present the following confirmatory evidence of a homologous relation to the male prostate and their control by the male hormone. Further, the reaction of these glands to the male hormone alone (as far as is known) distinguishes them as a biologic indicator for the presence of the male hormone in the female.

A series of 64 mature and 32 immature female rats were divided into 4 groups which received as follows: (a) 500 γ of testosterone acetate* or testosterone propionate,* (b) 500 γ of oestrone,* (c) 500 γ of testosterone acetate and 500 γ of oestrone, (d) only the oil vehicle. Therefore, rats in groups (a) and (b) were given, respectively, equal amounts by weight of the male hormone, testosterone acetate, and the female hormone, ketohydroxyoestrin, whereas rats in group (c) were given both male and female substances. Both hormones were dissolved in the same quantity (3 mg./cc.) of the

* Aided by a fund provided by Doctor George Walker.

1 Virchow, R., *Arch. f. path. Anat.*, 1853, **5**, 403.

2 Tourneux, F., *Compt. rend. Soc. de biol.*, 1888, **40**, 81. Evatt, E., *J. Anat. and Physiol.*, 1910, **45**, 122. Sachs, O., *Wien. klin. Wchnschr.*, 1911, **24**, 1420. Johnson, F., *J. Urol.*, 1922, **8**, 13.

3 Pallin, G., *Arch. f. Anat. n. Entwickel.*, 1901, p. 135.

4 Marx, L., *Z. Zellforsch. u. mikr. Anat.*, 1932, **16**, 48.

5 Price, D., *Am. J. Anat.*, 1936, **60**, 79.

6 Korenchevsky, V., and Dennison, M., *J. Path. and Bact.*, 1936, **42**, 91; **43**, 345.

7 Sachs, O., *Wien. klin. Wchnschr.*, 1911, **24**, 1420.

* Testosterone acetate and testosterone propionate were furnished through the courtesy of the Ciba Company; oestrone (theelin) through the courtesy of Parke, Davis and Company.

same oil (peanut oil), since the amount and type of oil influences the absorption.[8] All injections were made subcutaneously. Some animals were sacrificed on the 11th day; in other animals the administration was continued for as long as 3 months.

Results. From embryological studies by Price[5] and from experimental stimulation by injections of male hormone by Korenchevsky and Dennison[6] and by the present authors it is apparent that the female prostatic glands are homologous to male prostatic glands and are located caudal to the bladder in an urethral position similar to that of the ventral portion of the prostate in the male animal. Grossly, the glands resemble the male prostate. They present a pale flesh-pink color, a stippled surface and contain a clear, watery secretion similar to that of the male prostate. The glands are in the form of 1 or 2 disc-like lobes, which may reach a weight of 70 mg. in a single animal. Either a bilobular or unilobular condition may be present, the latter being more common with the lobe lying on either the right or left side of the urethra. In the bilobular condition one lobe lies on each side of the urethra. No opening from the glands into the urethra has been seen in serial section of the glands and urethra, but more material must be studied before denial is made of an outlet of the glands.

Histologically, the gland is composed of prostatic-like alveoli which are distended with fluid and lined with either cuboidal or columnar epithelium. Near the periphery of the lobe the alveoli are relatively small; they are lined by a tall columnar epithelium which is in some instances thrown into folds. The epithelium on the apex of these invaginations is usually pseudostratified. Alveoli in the more central portion of the lobe are commonly more distended with secretion with lining cells of a cuboidal type. The cells lining the alveoli save those of the low cuboidal type present a light-staining area in the cytoplasm distal to the nucleus similar to that described in the prostate. The nuclei are light-staining, vesicular, and proximally placed. The interalveolar spaces contain a few smooth muscle fibers surrounding the individual alveoli and considerable connective tissue, which is relatively more abundant near the periphery of the lobe.

The incidence of glands visible to the eye was low (9.4%) in 35 untreated virgin females; the few glands found were in young animals. In spayed animals and in those receiving oestrone Skene's glands were not observed. In the 48 animals receiving male hormone, the incidence was 58.3% (Table I).

[8] Parkes, A. S., *Lancet*, 1936, **2**, 674.

TABLE I.
Summary Table of Macroscopic Observations on Incidence of Skene's Ducts in Control and in Hormone Injected Rats.

Treatment	Paraurethral Glands		% Present
	Absent	Present	
Testosterone acetate or propionate 500 gammas daily—10-39 days	15	23	60.5
Testosterone acetate and ketohydroxyoestrin 500 gammas of each	5	5	50.0
Ketohydroxyoestrin 500 gammas	13	0	0.0
Control	32	3	9.4

The incidence of the paraurethral glands may be much higher than indicated by the figures in Table I, for counts included only macroscopically visible lobes. In the absence of routine histologic study and in view of the limited number of animals, these percentage figures are tentative and will probably need revision.

Skene's ducts are not only the embryologic homologue of the male prostate, but as previously indicated, also respond to the male hormone; they are not stimulated by oestrone but in either spayed or normal animals develop after male hormone administration into structures grossly and histologically similar to the male prostate.[6] The growth of Skene's ducts in response to male substances is in agreement with studies[10, 11] demonstrating the masculinizing or androgenic influence of male hormones in the female, particularly on those organs (clitoris, preputial and paraurethral glands) whose counterparts in male animals respond vigorously to male hormone.

The embryologic similarity of these glands to the prostate and their development in animals injected with male hormone but not in those injected with female hormone are evidence of the dependence of these structures upon the male substance. It is suggested that Skene's ducts of the rat may be of use as an indicator in the female of the effect of injections of male substance.

The occasional finding of well-developed glands in a normal uninjected female may be interpreted tentatively as indicative of the presence of male hormone-like substance in normal female animal under certain conditions. Further evidence that a substance similar to the male hormone may be produced in normal females is shown by recent studies[12] in which injection of large amounts of A.P.L.

9 Moore, C. R., Price, D., and Gallagher, T. F., *J. Anat.*, 1930, **45**, 71.
10 Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 22.
11 Hamilton, J. B., unpublished data.
12 Wolfe, J. M., *Anat. Rec.*, (in press).

into normal male or female rats, simultaneously with oestradiol resulted in a partial suppression of the reaction of the anterior lobe to oestradiol, a result which has since been duplicated by injecting male hormone simultaneously with oestrone.[13]

Summary. 1. Skene's ducts in the female rat are usually rudimentary. Male hormone substance stimulates growth of these glands to a condition which resembles grossly and histologically the prostate of the male rat. 2. In the present series the incidence of these glands in 35 normal animals is 9.4%, in 13 oestrone-injected animals 0%, in 48 male hormone-injected animals 58.3%. 3. The state of Skene's ducts may be utilized as an indicator of the presence of male hormone. 4. In view of the growth response to male but not to female hormones, the occasional finding of Skene's ducts in an uninjected female rat suggests the presence of male hormone in the normal female. 5. The growth response of the female prostatic glands to the male hormone is further evidence of the masculinizing effect of male hormone substance of the female animal.

9274

The Localized Sanarelli-Shwartzman Phenomenon in the Rabbit Kidney.

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A new phenomenon of tissue reactivity to bacterial filtrates has recently been described. The filtrates of many bacteria, hitherto not considered to produce exotoxins, have been shown to exert a marked toxic effect when 2 injections into a rabbit, the second injection being intravenous, are separated by an interval of 24 hours. The generalized reaction resulting when the first, or "preparatory" injection is given intravenously was described by Sanarelli,[1] and studied in detail by Apitz,[2] Gratia and Linz,[3] and Gerber.[4] The localized reaction resulting when the preparatory injection is given

[13] Wolfe, J. M., and Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 55.

* Research Fellow in Medicine, Harvard University.

[1] Sanarelli, *Ann. de l'Inst. Pasteur*, 1924, **38**, 11.

[2] Apitz, K., *Virchow's Arch. f. path. Anat.*, 1934, **293**, 1.

[3] Gratia, A., and Linz, R., *Ann. de l'Inst. Past.*, 1932, **49**, 131.

[4] Gerber, I. E., *Arch. Path.*, 1936, **21**, 776.

intracutaneously was described by Shwartzman,[5] and subsequently by numerous other investigators. The present status of the phenomenon is summarized and discussed by Shwartzman, Klemperer, and Gerber.[6]

Localized reactions in numerous organs have been described when the preparatory injection was given into the parenchyma of the organ, or even when the organ was prepared by way of its local vascular supply (bibliography in ref. 6). Among the organs described as having been made the site of local reactions is the kidney. Shwartzman[7] clamped the left renal vein in rabbits, injected 0.5 cc. of *B. typhosus* filtrate into the left renal artery and released the vein 5 minutes later. The "provocative" intravenous injection was made 24 hours later, and after 48 hours the prepared kidney showed "severe hemorrhagic lesions in the cortex and medulla." No gross lesions were seen in the opposite kidney, and no microscopic sections were made of either kidney. Loi and Cardia[8] reported similar lesions when a preparatory injection of 0.3 cc. of *B. typhosus* filtrate was made directly into the parenchyma of a rabbit kidney which had been "denervated" by stripping surgically the renal capsule and vessels.

The present study was made for the purpose of investigating the possible use of the localized renal phenomenon in the production of experimental nephritis. Both parenchymal and local intravascular preparation of the kidney were attempted.

The bacterial filtrate used was prepared from *B. coli* communis by the method of Shwartzman.[9] For intravascular preparation, the following technique was used: Under paraldehyde anesthesia the left renal artery and vein were occluded by kinking each separately over the point of a probe, and an injection of 0.2 to 2.0 cc. of filtrate was made into the distal portion of the renal artery. Both vessels were held closed for 3 to 5 minutes. The artery was then released, the vein punctured, and the kidney "washed out" by the removal of 3 to 8 cc. of blood from the renal vein, which was then released. For preparation by direct parenchymal injection, the needle was inserted through the abdominal wall into the substance of the left kidney, and 0.2 to 0.5 cc. of filtrate injected without anesthesia. In

[5] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 207; *J. Exp. Med.*, 1928, **48**, 247.

[6] Shwartzman, G., Klemperer, P., and Gerber, I. E., *J. Am. Med. Assn.*, 1936, **107**, 1946.

[7] Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.

[8] Loi and Cardia, *Boll. Soc. ital. di Biol. sper.*, 1934, **9**, 775.

[9] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 843.

all cases, a control injection of 0.2 cc. of filtrate was made in the abdominal skin at the time of the preparatory injection for the production of a localized Shwartzman phenomenon as a check on the susceptibility of the animal. Twenty-four hours later, 1 cc. of filtrate was given in the marginal ear vein.

Results. Intravascular preparation: Two control animals were given injections of 1 cc. of 0.4% phenol in normal saline into the left renal artery, and 2 more were given 1 cc. of *B. coli* filtrate in the same manner. No gross lesions were evident in any of these animals, and all the kidneys were normal histologically, when autopsy was performed 48 to 72 hours later. Five animals were then subjected to the intravascular preparation as outlined in the previous paragraph. Of these, one showed a negative control skin test, and at autopsy 12 days later all organs were grossly normal, and microscopic sections of liver, heart and both kidneys were normal.

The 4 animals showing positive skin tests were killed and autopsied 4 to 13 days after operation. The kidneys of all showed varying degrees of bilateral focal tubular necrosis in the convoluted tubules, less marked in the longer surviving animals, and 2 showed gross infarctions in the left kidney. There was a conspicuous absence of glomerular and other vascular lesions and of hemorrhage. The livers uniformly showed focal mid-zonal necrosis in various stages of fibrosis, and the myocardium in three showed fibrosing focal necrosis of muscle fibers. One animal developed a bloody diarrhea after 5 days, the intestine showing a severe non-specific inflammatory process microscopically.

Direct preparation of the renal parenchyma: Seven animals were used. In 2, the control skin tests were negative. Autopsies at 8 and 13 days respectively showed all organs grossly normal and both kidneys of each microscopically normal. In 5 animals, the control skin tests were positive. Autopsies from 6 to 13 days later showed in all the same bilateral focal necrosis of convoluted tubules in the kidneys, less marked in the longer surviving animals, that was seen in the animals prepared by way of the renal artery. No glomerular or vascular lesions were seen. In 3, the probable injection site in the left kidney was found, consisting of a sharply localized zone of bloodless glomeruli, dilated tubules and fibrosis extending from the capsule to fairly deeply in the cortex. Gross examination of the remaining viscera showed small focal necroses in the livers of 3, and in the heart of one, the latter proved microscopically.

The infarctions of the kidney with intravascular preparation and the sharply localized damage at the puncture site with direct paren-

chymal preparation may be fairly dismissed as the result of experimental trauma. In both series of experiments there remain the disappearing focal necroses of renal convoluted tubules, and the fibrosing focal necroses of liver and myocardium. These are undoubtedly later stages of the lesions described by Apitz and Gerber, and it would seem that because of the extreme vascularity of the kidney with anastomoses to surrounding vascular systems, sufficient bacterial filtrate escaped into the general circulation to prepare the entire animal for a generalized reaction in spite of the utmost care to restrict the reaction to the kidney. In the light of these results, it seems doubtful that the production of a localized Sanarelli-Shwartzman reaction in the kidney is possible.

The present author has observed small focal renal hemorrhages in the gross in animals dying a short time after the provocative injection, but these animals were discarded in favor of those surviving long enough to show late lesions at autopsy. The part played by the kidneys in a general reaction seems to have been a minor and transitory one, at least in animals that survived, compared to that played by the liver and myocardium, and in one case, the intestine.

Summary and Conclusions. 1. The production of a localized Sanarelli-Shwartzman phenomenon in the rabbit kidney was attempted, both by local vascular and direct parenchymal injection of *B. coli* filtrate. In neither instance was it possible to restrict the reaction to the kidney. 2. The results suggest that sufficient filtrate entered the systemic circulation from the preparatory injection to prepare the entire animal for a generalized reaction, and make it doubtful that the production of a localized renal phenomenon is possible. 3. The late lesions of the generalized phenomenon are described. These seem to indicate the relative insignificance of the renal lesions in surviving animals as compared to those of the liver and the myocardium.

9275 P

Influence of Diet on the Course of Nephrotoxic Nephritis in Rats.

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While numerous investigations concerning the effect of diet on Bright's disease have been reported, the laboratory approach to this problem has been criticized on the ground that experimental nephritis usually fails to correspond closely with the disease as it occurs in man. The chronic nephritis induced by Masugi[1] in rabbits with anti-kidney serum has reawakened a general interest in nephrotoxins; and with suitable nephrotoxic sera we[2, 3] have induced nephritis in rats, which, in clinical course, disturbance of renal function, and development of pathological picture, resembles human Bright's disease closely enough to permit it being used as a tool for evaluating the effects of diet on the course of nephritis.

Forty-eight young rats were fed a purified diet while renal function studies, plasma protein and hemoglobin determinations, and urinalyses were made by the methods previously employed.[2, 4] Then severe nephritis was induced uniformly in all animals by intravenous injections of anti-kidney serum given on 3 successive days. The animals were divided into 3 comparable groups and fed isocaloric diets. Each diet contained 27% fat, 4% salt mixture and vitamins with the following variations: diet No. 1, 64% carbohydrate and 5% protein; diet No. 2, 51% carbohydrate and 18% protein; diet No. 3, 29% carbohydrate and 40% protein. Lactalbumin served as the protein, Crisco as the fat, and dry Karo powder with cane sugar as the carbohydrate. Observations were continued for 11 months after injection of nephrotoxic serum. Urea clearance determinations and plasma protein values were obtained every third week, hemoglobin values at 3- to 6-week intervals and urine analyses and body weights were recorded weekly.

The course of the nephritis in all 3 groups was parallel for the first month; severe albuminuria and cylindruria appeared and persisted, anasarca was present for from a few days to several weeks; plasma protein values were temporarily depressed; neither the blood

[1] Masugi, M., *Beitr. path. Anat. u. allg. Path.*, 1933, **92**, 429.
[2] Smadel, J. E., and Farr, L. E., *J. Exp. Med.*, 1937, **65**, 527.
[3] Smadel, J. E., *J. Exp. Med.*, 1937, **65**, 541.
[4] Farr, L. E., and Smadel, J. E., *Am. J. Physiol.*, 1936, **116**, 349.

urea nor the urea clearance was significantly altered except in those animals (one in each group) that succumbed during the acute phase.

In the second month evidences of nephritis diminished greatly or disappeared in all but 2 animals on diet No. 1 (low protein). The sudden death of these 2 rats in the fifth month, without antecedent renal failure, seemed attributable to severe acute liver necrosis of unexplained origin. Eight and a half months after injection none of the 13 surviving rats fed diet No. 1 had elevated blood urea or depressed urea clearance values; moreover only one animal had urine abnormalities of even moderate degree. At this point 5 of the apparently recovered rats were changed from diet No. 1 to diet No. 3 and, in the ensuing 2 months, 3 of the animals developed moderate albuminuria with some casts, but neither the urea clearance, blood plasma nor hemoglobin were depressed. Growth was retarded in the animals on diet No. 1 and was never as great as in rats on the other diets.

Every animal fed diet No. 3 developed progressive nephritis. Only 2 animals, both in terminal phases of the disease, were still alive when the experiment was stopped; the rest died of apparent renal failure, averaging 6 months survival after injection.

Of the rats fed diet No. 2, one recovered completely during the second month, 6 had abnormal urinary findings throughout the period; 8 of the 15 rats that survived the acute phase of the disease died of apparent kidney failure; the time of death averaged 5½ months after the initial injury.

Diagnosis of renal failure was based on progressive fall of the urea clearance and rise in the blood urea levels. Terminally the majority of the rats with progressive nephritis showed also anemia, polyuria, and weight loss.

Pathological lesions found in nephritic rats fed diet No. 2 were practically identical with those previously described[3] in rats with this type of nephritis kept on an ordinary mixed diet. Renal scarring was more intense in the group fed diet No. 3 while the rats that received diet No. 1 and recovered clinically had slight renal damage represented by old scarring. Generalized vascular lesions resulting in secondary degenerative changes, especially in the heart, occurred in most of the animals with progressive nephritis.

Conclusions. 1. Under certain conditions chronic progressive nephritis follows a *single* insult to the kidney. 2. The course of nephrotoxic nephritis in rats can be markedly influenced by diet.

9276

Studies on Alum-Precipitated Insulin.

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The precipitation of active biological principles, such as diphtheria toxoid, by alums suggested to us the possibility of preparing an alum-precipitated insulin. After trial of several preliminary procedures, we adopted the following technic:

To 100 cc. of ordinary insulin U40 is added 10 cc. of a 50% (by weight) solution of $NaAl(SO_4)_2.12\ H_2O$ in sterile distilled water. A heavy turbidity forms instantly and, upon mixing, a copious precipitation occurs. After standing for 24 hours in the refrigerator at 5°-10°C., the mixture is centrifuged at 3500 r.p.m. for 30 minutes. The clear supernatant fluid is now decanted from the sediment. Bioassay of this fluid, performed in the usual manner on rabbits, shows that it contains only 5-10% of the original insulin unitage.

To this supernatant fluid is now added a further portion of 10 cc. of 50% soda-alum solution. After remaining for one week in the refrigerator at 5°C., a second crop of precipitate is obtained. This crop is separated by centrifuging from the supernatant fluid which now proves insulin-free by bioassay.

The precipitate obtained by the action of alum on insulin solutions, hereinafter referred to as alum precipitated insulin, is a white amorphous powder, insoluble in alcohol, ether and petroleum ether and slightly soluble in neutral (pH 7) distilled water. It is readily soluble in undiluted serum, but only slightly soluble in serum diluted 1:5 with saline. In solutions of acidity greater than pH 2.5, it dissolves completely. In solutions of pH 2.5 to 7, it forms insoluble suspensions, the minimum solubility being obtained in solutions of pH 6.

On dissolving separately the 2 precipitates in solutions of pH 1 to pH 2 and assaying the resultant clear solutions on rabbits, it is found that the first precipitate contained 90-95% of the total original insulin unitage. The second precipitate contained the remaining 5-10%. Thus, we find that a quantitative recovery of the active principle from insulin solution may be effected by fractional alum precipitation.

Chemical analysis of the precipitate showed a concentration of 0.10 to 0.12 γ of aluminum and 4.0 to 7.0 γ of non-protein nitrogen per unit of insulin.

For experimental and clinical trial, the precipitate of 100 cc. of insulin U-40 was suspended in 50 cc. of sterile distilled water containing 0.1 cc. N/10 HCl per liter, to which has been added 0.2% of phenol. This suspension now assays 80 units of alum-precipitated insulin per cc.

Hypoglycemic activity of the suspension was studied in the following manner: A series of 44 rabbits weighing between 1.72 and 2.28 kg., which had been starved for 24 hours, were injected subcutaneously with a suspension of 3 units of the alum-precipitated insulin. In view of the relative uniformity of our results, Table I is limited to the blood sugar levels observed in series of 6 rabbits.

TABLE I.

Animal No.	8	11	14	18	24	30	38
			Blood Glucose.				
Fasting	168	148	111	122	129	234	184
hr.							
1	158	149	108	118	126	240	172
2	152	154	107	119	124	220	170
3	148	139	101	111	122	194	166
4	140	138	101	108	121	186	154
5	128	137	88	102	108	172	120
6	129	135	86	99	104	170	111
7	111	132	74	94	101	162	98
8	108	120	70	80	94	140	88
9	101	111	68	62	84	131	74
10	98	101	62	54	62	128	70
11	98	108	50	55	59	124	64
12	110	120	52	53	60	126	64
13	114	124	56	68	62	128	68
15	119	128	59	84	64	134	79
17	128	123	72	90	69	168	101
19	162	129	88	92	88	170	138
21	158	140	98	97	111	172	164
23	164	144	108	101	120	168	158
25	165	158	112	124	134	184	160
26	163	156	113	125	130	170	166

These results show that 3 units of alum-precipitated insulin cause a blood glucose depression in rabbits of 62 to 110 mg. % in 7 to 11½ hours, with recovery to the preprandial glycemia in 15 to 24 hours. Hypoglycemic tetany (insulin shock) was evident in no case.

Three days after recovery from the alum-precipitated insulin, each of the animals in the above series received injections of 3 units of ordinary insulin. The maximum depression of blood sugar was 74 to 122 mg. % in 1 to 2 hours, with insulin shock in all 6 cases.

It is not within the scope of this paper to discuss the therapeutic action of alum-precipitated insulin, which is still under clinical in-

vestigation. However, a few typical data of its hypoglycemic effect on human diabetics are here presented.

Five patients with diabetes who had been attending the hospital dispensary for a number of years and whose diet and insulin requirements had been established previously, received in one morning injection, a unitage of alum-precipitated insulin equivalent to the total number of units of ordinary insulin previously administered in 3 or 4 daily injections. The patients were kept on their usual diet in the course of these observations. Table II illustrates their blood glucose fluctuations during the day.

TABLE II.

Patient Injected	♀ Aet 45 150 U	♂ Aet 29 100 U	♂ Aet 52 160 U	♀ Aet 52 160 U	♀ Aet 29 100 U
	Blood Glucose Concentration.				
Time hr.					
1	264	212	328	312	234
2	278	244	310	308	212
3	256	210	288	290	208
4	221	186	248	288	196
5	186	178	231	246	208
6	174	172	234	240	198
7	148	130	230	227	182
8	138	122	212	181	140
9	122	104	186	148	121
10	118	105	142	130	111
24	246	196	322	302	212
25	258	210	329	308	215
26	270	208	312	318	220

This table indicates that alum-precipitated insulin injected into human diabetics causes a maximum blood glucose depression in 7½ to 12½ hours, with recovery to the preprandial level in 15 to 30 hours.

We may assume, therefore, that alum-precipitated insulin has, in comparison with ordinary insulin, a delayed and prolonged hypoglycemic action on experimental animals, as well as human diabetics.

9277

Chemical Nature of the Reynals Spreading Factor from Mammalian Testicle.

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The properties of the spreading factor found in aqueous extracts of mammalian testicle have been recently reviewed by McClean[1] and by Duran-Reynals.[2] This factor causes an increased permeability of the dermis to inert particles such as dyes and India ink, also to bacterial toxins, bacteria and viruses. How this effect is produced is not clear. It has been attributed to an alteration of the connective tissue barrier and some changes have been found in the histological appearances of the collagen fibers. It does not appear to be caused by an increased flow of lymph; the property can be demonstrated in excised skin as long as 48 hours after death. Aside from mammalian testis, the "spreading factor" has been demonstrated in other tissues, such as brain, liver and kidney, but in relatively small amounts. It is present in considerable quantity in certain bacteria, in certain tumors, in certain snake venoms and insect venoms; the factor is closely associated with the venom but can be dissociated from it.

Attempts to ascertain the chemical nature of the spreading factor have been made along two lines. A great variety of pure chemical substances have been examined for spreading properties, in most cases with negative results. Some spreading action is found in the case of glycerine, triacetin, with certain lecithins and with some commercial peptones. The most encouraging observation along this line has been with azoproteins. Various chemical studies have been made upon the testicular extracts which have thrown some light upon the chemical nature of active agent, and have suggested that it is more closely related to proteins than to lipoids or carbohydrates: The active principle is precipitated by ammonium sulphate, by basic lead acetate, by alcohol and ether, and by acetone. It is soluble in water and in dilute acids. It is relatively thermostable, resisting 100°C. for several minutes in the moist state and higher temperatures when dry. It passes through a

* Commonwealth Fund Fellow in Biochemistry.

1 McClean, D., *Biol. Rev.*, 1933, **6**, 345.

2 Duran-Reynals, F., *Ann. de l'Inst. Pasteur*, 1936, **57**, 597.

Berkefeld filter, but is apparently non-dialysable. It is inactivated by trypsin; however, autolysis of certain tissues like brain in which it is present in small quantity, causes an increase in the concentration. Although purified preparations have been made which are active in a dilution of 1:1,000,000, none of these products have been protein-free. The experiments here reported were undertaken to confirm and extend knowledge of the chemical properties of the spreading factor.

For the purification of the extract the method suggested by Claude and Duran-Reynals was used. Minced testicles were extracted with n/10 acetic acid, the filtered extract being precipitated by acetone.† The acetone precipitate was dried, dissolved in water, filtered and reprecipitated by acetone, the process being repeated 10 times in all. The various acetone precipitates were studied independently, being tested for potency by observing their effect on the spread of a suspension of India ink in physiological saline or m/50 phosphate buffer (pH 7.1) injected intradermally into the flank of rabbits. Control injections were made in all instances.

The first acetone precipitate (yield 21.0 gm. per kilo of testicle) was a light brown powder, not completely soluble in water. The second acetone precipitate (yield 2.6 gm. per kilo of testicle) was colorless and almost completely water soluble. A slight amount of suspended material was present which could be removed by a Seitz No. 3 filter. Subsequent precipitates were similar in character and showed very little diminution in yield and no perceptible gain in potency. Most of the work described below was done with the second and third acetone precipitates.

Preliminary tests were done which confirmed data in the literature as to the properties of the spreading factor. In powdered form it was stable at room temperature, but solutions deteriorated rapidly unless kept in the icebox. On heating the solutions to 100°C. for 5 minutes, potency was completely lost by the first acetone precipitation (apparently because of adsorption by the large amount of protein coagulated by this procedure) but the more purified products when subjected to this treatment yielded negligible coagulation and retained their potency.

The extracts were soluble in concentrated KOH and in glacial acetic acid in the cold. With strong mineral acids the material went into solution only on warming with the development of colors (violet with HCl, pink with H_2SO_4; deep yellow with HNO_3).

† This first step of the process was carried out for us by the research laboratory of E. R. Squibb & Sons, through the courtesy of Dr. John F. Anderson.

When dissolved in distilled water the acetone precipitates yielded solutions with a pH between 6.2 and 6.9; the solutions were well buffered.

Qualitative tests for carbohydrates, lipoids and proteins were carried out. A slight alpha naphthol test was given by all the active fractions, and after hydrolysis with HCl the solution showed a slight reducing action. Thus small amounts of carbohydrate may be present—free or in combination. The precipitates were insoluble in fat solvents, but in order to exclude the possibility of lipoid complexes being present, material was treated with alcoholic KOH, acidified and then extracted with ether. A negligible amount of material (inactive) was found in the ethereal extract. The Xanthoproteic, Millon's, Hopkins-Cole glyoxylic acid reaction and diazo reactions were all positive; a weak biuret test was obtained. The active material was precipitated from solution by protein precipitants: ammonium sulphate, mercuric chloride, trichloracetic acid and lead acetate. Thus, in confirmation of previous work, it appears that the active material is at least intimately associated with protein.

Elementary analyses of the first, second and third acetone precipitates were made‡ in the hope of demonstrating a shift in composition coincident with purification:

	C	H	N	S	P	Ash
First Acetone Precipitate	44.50	6.42	12.31	0.92	2.24	11.70
Second " "	37.99	5.87	11.63	0.83	3.81	20.00
Third " "	38.33	5.86	11.47	0.98	3.89	19.73

When calculated on an ash-free basis the analyses show somewhat lower C and N percentages than are obtained for typical proteins, but they fail to show any consistent shift in composition with purification. The most striking change is the increased percentage of ash. All preparations gave a test for inorganic phosphorus. Only a slight direct test for sulphur was obtained with sodium nitroprusside, but after fusion with sodium and extraction with water, a definite positive test was obtained indicating that most of the sulphur was in organic combination.

An attempt was made to see whether the active material could be inactivated by X-rays or ultraviolet rays. A 1% solution of second acetone precipitate (filtered through Seitz No. 3 filter) was exposed for one hour, being given 9000 roentgen units. At the end of this

‡ For the C, H, N and S analyses we are indebted to Prof. Hans T. Clarke of the Dept. of Biological Chemistry, Columbia University, in whose laboratory they were performed.

time it was unchanged in appearance and showed no loss in activity. A similar solution in a shallow pan was exposed for ½ hour to a mercury quartz arc at a distance of 10 cm. At the end of this time a precipitate had formed. Both precipitate and solution were completely inert.

Although it has been reported that the Reynals factor is not dialyzable, it appears that dialysis experiments were not carried out at varying hydrogen ion concentrations. We prepared 1% solutions which were placed in parlodion bags and dialyzed for 48 hours against citrate buffer solutions of pH 3, 5 and 8. The external medium was changed every 12 hours. In no case did active material appear to pass through the membrane. In a further experiment dialysis was carried out against a continuous flow of water. The material inside the membrane retained its activity. It thus appears that the Reynals factor either has a high molecular weight or is intimately associated with material of high molecular weight.

An attempt was made to study the behavior of the spreading factor in an electric field. A 5% solution of the second acetone precipitate was placed in a U-tube and a current of 5 milliamperes was passed through for 24 hours. At the cathode a brown protein-containing precipitate formed which settled to the bottom of the tube; it was found to be almost completely inactive. The solution at the cathode was tested after neutralization and was found to have lost the greater part of its activity. The solution at the anode, however, after neutralization was found to possess all the activity of the original solution. It thus appeared that the active material either was negatively charged and migrated to the anode or else was inactivated at the cathode by the precipitation of protein which took place there.

We then attempted to purify the active material by electrodialysis. A 5% aqueous solution of the third acetone precipitate was placed in the central portion of a Bradfield 3-chamber electrodialysis cell. Distilled water was placed in the 2 outer compartments containing the electrodes. The chambers were separated by parlodion membranes. A current of 10 milliamperes was passed through for 24 hours. No obvious change occurred in the outer chambers, but in the center a white precipitate settled out. The supernatant fluid remained a pale yellow color. At the end of the experiment the precipitate and the fluid in the 3 compartments were tested for activity. The precipitate and the fluid in the outer compartment containing the electrodes were inactive, whereas the clear fluid in the central compartment retained all the original activity. The precipitate appeared to be protein in nature, and its formation is

apparently due to a decrease in solubility caused by the passage of electrolytes into the lateral chambers containing the electrodes.

Further purification of the liquid in the central compartment was attempted by putting fresh distilled water in the outer chambers and passing the current through again. A further precipitate then settled out which on testing proved to be inactive. The supernatant fluid had retained its full activity. On evaporation of the supernatant fluid a solid was obtained which represented only 1/10 of the original material and contained only a very small percent of ash. Elementary analyses of this material are in progress.

Conclusion. Electrodialysis appears to be a very promising method for the purification of the Reynals spreading factor.

9278 P

Inhibition of Estrous Cycle in the Rodent with Post-partum Urine and Commercial Prolactin.

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In attempting to ascertain the effect of urine from lactating women upon mammary glands of rats and mice, it was observed that there was an inhibition of estrus in these animals. Animals with normally recurring estrus were used, and these had daily vaginal smears for at least 3 consecutive cycles as a means of control. Injections of post-partum urine were then started. It was found that at least one, usually 2, and occasionally 3 cycles were suppressed, after which the animal resumed its normal estrous rhythm in spite of continuation or increase in the amount of injected urine. Controls, injected with the urine of normal human males and females had no alteration of the rhythm. The rodent's ovaries during this period of induced diestrus contained active corpora lutea which were similar to the ovaries of lactating animals. If injections were discontinued during this period of induced diestrus, a vaginal smear indicative of estrus was obtained within 48 hours. These findings suggested that a sub-

* Lucius N. Littauer Fellow in Cancer.

stance, possibly prolactin, was present in the urine of lactating women, which accounted for the amenorrhea and which could produce analogous changes in rats and mice. Since Lyons and Page[1] reported the isolation of prolactin from the urine of lactating women, extracts were made from such urine, using their method. Only suggestive inhibition was obtained with this preparation.

Commercial prolactin prepared according to the method of Riddle was obtained through the courtesy of E. R. Squibb and Sons. Normal adult female mice in which the rhythm of the cycle was determined for at least 3 periods of estrus were used. These preparations were also capable of inhibiting the estrous cycle but for a longer time than did the post-partum urine. This confirmed the work of Dresel[2] and Lahr and Riddle,[3] which was reported during the course of these experiments. The former obtained suppression of the estrous cycle in mice whereas the latter investigators found a similar phenomenon in rats after the injection of prolactin.

The following are the preliminary observations from this laboratory: (1) Three bird units of prolactin given in divided doses twice daily was the minimal amount which would inhibit the estrous cycle for at least 3 periods. This was a constant finding although Dresel reported the suppression with smaller amounts. Smaller dosages as a rule had no effect upon the rhythm. (2) Larger doses seemed to suppress the cycle in proportion to the size of the dose, *e. g.,* 8 B.U. divided and given twice a day inhibited 4 or 5 cycles and 16 B.U. given in the same manner generally prolonged the period of diestrus for at least 40 days. With the latter dosage, the animal not infrequently failed to resume its estrous cycles even though injections were discontinued at the end of 40 days. (3) Lahr and Riddle[3] observed that about 15 days of diestrus were sometimes followed by estrus and then another period of prolonged diestrus. They further ascertained that discontinuance of injections resulted in estrus within 48 hours. These findings were confirmed in these experiments. It is possible that the former phenomenon is similar to the period of pseudo-pregnancy seen in the rodent. It was further determined that the appearance of estrus after the injections were stopped corresponded to the time it should have occurred

[1] Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **32**, 1049.
[2] Dresel, I., *Science,* 1935, **82**, 173.
[3] Lahr, E. L., and Riddle, O., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 880.

during the normal rhythm of the animal if it were left untreated. (4) Sections of the ovaries after a short period of inhibition (1 to 3 weeks) revealed active corpora lutea, which were also found by Lahr and Riddle. Sections after prolonged diestrus (4 to 8 weeks), however, showed atrophy and degeneration of the ovary. This probably explains the failure of an animal to resume its rhythm even though injections were discontinued. In view of the presence of corpora lutea a search was made for a substance other than prolactin such as the luteinizing hormone (LH) of the hypophysis which could be responsible for the changes described. Consequently, the commercial preparations were assayed by one of us (H.L.F.) and sufficient LH was found to initiate luteinization of the follicles. A substance similar in its action to LH was then isolated from the urine of lactating women (10 days to 3 months post-partum). Injections of LH (prepared by H.L.F.) containing only small quantities of lactogenic hormone produced more striking suppression of the cycles. The ovaries, microscopically, contained many more active corpora lutea than those found after prolactin injections. It is interesting that recently an extract of LH entirely free from prolactin has been obtained for the first time (H.L.F.). Experiments being carried out at present with the new preparation will be reported later.

It seems, as a result of the aforementioned experiments, that the causative agent in the inhibition of the estrous cycle in the rodent is LH and not prolactin although this needs conclusive proof. These experiments suggest also that LH or a similar substance produces the amenorrhea of lactation rather than prolactin. This would correspond more closely with the so-called corpus luteum of lactation.

9279

Relation of Reticulo-Endothelial System to Refractoriness Developed in Response to Gonadotropic Hormone.

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We have presented evidence[1] to show that the so-called reticulo-endothelial system is intimately concerned with the development of refractoriness in rats to gonadotropic substance. We demonstrated that, whereas the ovaries of normal immature female rats, in response to daily doses of 10 R.U. of pregnancy urine extract, reach a maximum size after 10-15 days of treatment and then regress, the ovaries of splenectomized littermate animals continue to grow, attaining weights approximately 2 to 3½ times that of the treated controls, 20-30 days after beginning of injection. This increase in weight was shown to be due almost entirely to an increase in the size and numbers of corpora lutea. The ovaries of the splenectomized animals begin to regress after 30 days despite continued treatment. The interpretation given for these results was that with the extirpation of the spleen a large portion of the reticulo-endothelial system concerned with the production of the inhibitory substance for the luteinizing principle present in the pregnancy urine extract has been removed; this would therefore result in larger ovaries but that soon the function of producing the inhibitory substance would be taken over by other reticulo-endothelial elements in the liver, lymph and haemolymph nodes, etc., which are known and shown in our results to become hypertrophied and activated following removal of the spleen.

In the experiments described above and those to be presented below, only animals free of infection, especially *Bartonella muris*, a latent disease in rats, were employed. This is absolutely necessary since infection is a factor affecting reticulo-endothelial activity.

In order to further test our hypothesis that the activity of the reticulo-endothelial system is related to the loss of sensitivity following chronic treatment with heterozoic endocrine extracts, we have made a comparison of the abilities of plasma taken from treated splenectomized and control rats to neutralize the effects of

[1] Gordon, A. S., Kleinberg, W., and Charipper, H. A., *Science*, (in press).

injections of hormone in test animals. Three splenectomized and 3 control immature female hooded rats were injected with 10 R.U. Follutein daily for 20 days. At the end of this time they were subjected to light ether anesthesia and the blood, drawn by cardiac puncture, collected in oxalate. It was then centrifuged and the plasma pipetted off. Fourteen immature female hooded rats were used for testing the plasma. Seven of these were given injections of 0.1 cc. plasma of the treated normal rats along with 5 R.U. Follutein daily for 10 days, whereas the other 7 received daily injections of 0.1 cc. of plasma from the injected splenectomized rats plus 5 R.U. Follutein for the same period of time. The extract was injected on one side and the plasma on the other side of each animal to avoid mixing. At the end of 10 days the animals were sacrificed. The weights of the ovaries of animals injected daily for 10 days with 5 R.U. Follutein plus 0.1 cc. serum from 20-day Follutein treated normal rats were as follows: 44 mg., 48 mg., 43 mg., 78 mg.,* 47 mg., 39 mg., 43 mg.; average weight, 49 mg. The weights of the ovaries of animals injected daily for 10 days with 5 R.U. Follutein plus 0.1 cc. serum from 20-day Follutein-treated splenectomized rats were as follows: 82 mg., 60 mg., 72 mg., 59 mg., 87 mg., 83 mg., 91 mg.; average weight, 76 mg.

Although the number of test animals is relatively small, the results do seem to indicate that the plasma of 20-day injected control rats has inhibited the injected hormone preparation to a greater extent than the plasma of similarly treated 20-day splenectomized animals. This would account for the smaller size of the ovaries in the former as compared to the latter group of animals.

Further experiments were then conducted to determine whether the property producing the inhibitory substances to injected heterozoic hormone extracts is peculiar to the spleen or is possessed as well by the reticulo-endothelial elements in other organs. A group of 6 immature hooded female rats were splenectomized and then injected for 20 days with 10 R.U. Follutein. Beginning with the 20th day, and every second day thereafter, they were given intraperitoneal injections of trypan blue in physiological saline along with continued daily doses of 10 R.U. Follutein. The purpose of this was to see whether it would be possible to "block" with the injected dye, at least to some extent, the reticulo-endothelial compensation which develops and becomes obvious in the treated animals 20-30 days after removal

* This animal was the only one in the entire series which showed, at autopsy, a very large congested spleen, a sign of *Bartonella muris* infection. In the majority of such animals large ovaries result in response to treatment, due most likely to "blockage" of the reticulo-endothelial system.[1]

of the spleen. The animals were then sacrificed at intervals following this treatment, and the weights of the ovaries compared with those of Follutein-treated splenectomized animals not injected with the dye. The results are given in Table I.

TABLE I.

Immature splenectomized rats injected daily with 10 R.U. Follutein plus 1 cc. 0.5% trypan blue beginning with 20th day and every second day thereafter.			Immature splenectomized rats injected daily with 10 R.U. Follutein.		
No. Days After Splenectomy	No. of Animals	Wt. of Ovaries	No. Days After Splenectomy	No. of Animals	Wt. of Ovaries
		mg.			mg.
28-30	2	210-245	27-30	6	160-265
34-36	3	295-377	34-37	4	114-158
46	1	593	45-50	4	62-91

It is seen from this table that the trypan blue treatment has not only prevented the regression in size of ovaries which occurs rather rapidly in Follutein-treated splenectomized animals after 30 days, but in addition has resulted in a further considerable increase in size for at least 25 days beyond this period.† All the ovaries of the dye-injected animals show an increase in the numbers of large corpora lutea. This is similar to the response of ovaries in Follutein-treated animals whose reticulo-endothelial elements are blocked by the Bartonella organism.[1] The most obvious interpretation of these results is that the dye has produced a "blockage", in the splenectomized animals, of the remaining compensating reticulo-endothelial elements which normally take over the function of the spleen in inhibiting the action of the injected hormone. The treated splenectomized animals, given repeated injections of dye, are most likely producing much smaller quantities of the inhibitory principle for the hormone than the Follutein-treated splenectomized animals not injected with the dye.

Summary. 1. Immature splenectomized rats injected with pregnancy urine extract for 20 days produce a smaller quantity of inhibitory substance than normal injected controls. 2. "Blockage" experiments with trypan blue indicate that the production of antagonistic principle is not peculiar to the spleen but is possessed by the reticulo-endothelial system as a whole.

† The mortalities become high after repeated injections of the dye because of an increased susceptibility to infection.

9280

Cystine in Normal and Cystinuric Human Blood.

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It is usually stated that cystine is not present in detectable quantities in normal human blood.[1] The failure of the satisfactory recovery of small amounts of cystine added to blood has been attributed, by Harding and Cary,[2] to difficulties in the preliminary deproteinization necessary prior to cystine determination. They have reported the presence of little, if any, free cystine in cow blood plasma. The amino nitrogen of normal blood (4-7 mg. %) should include small amounts of cystine nitrogen. If loss of cystine in deproteinization could be avoided and if a sufficiently delicate method for the determination of cystine were available, it should be possible to detect cystine in normal blood.

By the use of ultrafiltrates of oxalate-fluoride plasma, mechanical loss of cystine by deproteinization has been avoided and the application of the method of Sullivan to the determination of cystine in such ultrafiltrates has been made possible by the use of the Pulfrich photometer. The details of the preparation of the ultrafiltrates and the determination of cystine will be presented elsewhere in connection with studies of the intermediary sulfur metabolism of experimental animals. Recovery of small amounts of cystine added to blood has been satisfactory.

Application of the above procedure to normal human blood has indicated the presence of approximately 1.0 mg. % of cystine in plasma ultrafiltrates. Samples of blood of one of us (B.H.B.), taken after fasting periods of 12 to 18 hours, have given values of 0.71, 0.80, 0.92 and 0.93 mg. % of cystine in the plasma ultrafiltrate and values of 0.80, 0.91 and 1.05 mg. after meals. The ultrafiltrate of the blood of the same individual, withdrawn 3 hours after the oral administration of 6 gm. of cystine in gelatine capsules, contained 1.72 and 1.40 mg. % of cystine, respectively in 2 experiments. In a third study, in which 7 gm. of cystine were fed, 1.30 and 0.88 mg. % of cystine were present in the plasma ultrafiltrates of blood samples taken 3 and 5 hours later, respectively. In 4 other normal subjects, cystine values ranging from 0.82 to 1.13 mg. were obtained.

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1 Hess, W. C., *J. Wash. Acad. Sci.*, 1929, **19**, 491.

2 Harding, T. S., and Cary, C. A., Proc. Soc. Exp. Biol. and Med., 1926, **23**. 319; *J. Biol. Chem.*, 1928, **78**, xlix.

The plasma ultrafiltrate of the blood of a cystinuric patient (S.P.)[3] contained 1.13 mg. % of cystine, a value slightly higher than our average normal value, but, we believe, within the upper limit of the range of values for normal human blood. To this subject, 5.6 gm. of methionine were administered in 2 equal portions at the morning and midday meals. The plasma ultrafiltrate of a sample of blood withdrawn at 3:45 P. M. contained 0.92 mg. % of cystine. It may be noted that, while the isolation of cystine from cystinuric blood has been claimed,[4, 5] no quantitative data have been reported.

It should be noted that no attempt was made to determine the possible presence of cysteine in these ultrafiltrates. The values reported represent "total" cystine, *i. e.*, values obtained by the application of the Sullivan procedure to the ultrafiltrates after reduction with cyanide.

This research was made possible by a grant to one of us (H.B.L.) from the Faculty Research Fund of the University of Michigan for the study of cystinuria.

9281

Effect of Various Sterols on Thymus in the Adrenalectomized Rat.

JOSEPHINE SCHACHER, J. S. L. BROWNE AND H. SELYE. (Introduced by J. C. Meakins.)

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It has been pointed out that when an organism is seriously injured by an acute, non-specific, nocuous agent, a characteristic syndrome occurs, which consists of a rapid involution of the thymus and lymphatic system, oedema formation, with fluid in the pleural and abdominal cavities, loss of muscle tone, fall in body temperature, lung hemorrhages, and ulcers in the digestive tract. This syndrome has been given the name "Alarm Reaction".[1]

It has been shown further,[2] that, while adrenalectomy facilitates

[3] Lewis, H. B., Brown, B. H., and White, F. R., *J. Biol. Chem.*, 1936, **114**, 171.

[4] Demoulière, A., *La Cystinurie*, 1911, *Thèse pour le Doctorat en Médecine. Faculté de Médecine de Paris.*

[5] Müller, A., *Wien. med. Woch.*, 1911, **61**, 2364, 2488.

[1] Selye, H., *Endocrinology*, 1937, **21**, 169.

[2] Selye, H., *Br. J. Exp. Path.*, 1936, **17**, 234.

the production of all other symptoms of this "Alarm Reaction", it prevents the thymus involution. Physiological NaCl solution given to the animals restores their resistance to damaging influences, but does not enable the thymus to involute. Adrenaline is without effect both on the resistance of rats and on the ability of the thymus gland to respond to toxic stimuli. In animals maintained with very large doses of cortin slight involution will occur. This may be due to small amounts of some contaminating substance present in the cortin, or to the cortin itself. The only other substance which was found to be active in causing thymus involution following bilateral adrenalectomy was oestrone.[3]

The following investigations were carried out in order to determine whether the thymus of adrenalectomized rats would involute following the administration of sterol substances other than oestrone and at the same time to study the toxicity of these substances in such animals.

Rats varying in age from 40 to 162 days were used, 5 to 8 rats in each series with an equal number of controls. They were given standard Purina food and normal saline. Adrenalectomy was performed in one stage under ether anesthesia. Following a 24-hour rest period injections were begun.

The substances investigated were: 1. oestrone; 2. oestradiol; 3. pregnandiol; 4. dehydroandrosterone acetate; 5. cholestenone; 6. cholestenone acetate; 7. cholesterol; 8. progesterone; 9. androstenediol; 10. testosterone; and 11. testosterone propionate.* These compounds were administered in oil solution by daily subcutaneous injections. All the animals were autopsied for evidences of the alarm reaction, and the weights of the thymus, spleen and lymph nodes recorded. Survival was used as the criterion of toxicity.

Eight male rats were given 100 gamma of oestrone daily for 10 days. By that time half of the animals died. The remainder and the control animals were killed and autopsied. The oestrone produced a decided decrease in the size of the thymus, spleen and lymph glands.

Thymus Weights. Rats given oestrone: 256, 151, 293, 153, 185, 350, 237, 143; average 221. Controls: 580, 512, 514, 366, 460, 378, 495; average 472.

A group of seven 80-day-old females resisted the same dose of oestrone for 14 days. The thymus was even more markedly invo-

[3] Selye, H., Harlow, C. M., Collip, J. B., *Endokrinologie*, 1936, **14**, Heft 1/2.

* The authors are indebted to the Schering Corporation, who through the courtesy of Dr. E. Schwenk, kindly supplied most of these compounds.

luted, the average weight being 81 mg., the control rats averaging 322 mg.

Pregnandiol in daily doses of 100 gamma for 12 days had no toxic action and did not alter the thymus gland of 7 male rats. Daily doses of one mg. of pregnandiol for 17 days were also without effect in a series of 8 females.

Because of the similarity in structure between the 2 sterols, pregnandiol and oestrone, it was thought possible that pretreatment with the apparently inert pregnandiol would increase the resistance of the adrenalectomized rats to oestrone by stimulating the same detoxifying mechanism. The opposite was found to be the case. Seven rats were pretreated with pregnandiol, 100 gamma daily for 10 days. Seven control rats received injections of oil during this period. Following adrenalectomy the pregnandiol was discontinued and both groups were given 100 gamma oestrone daily, with the result that 5 of the pretreated group died after 7 injections of oestrone. Only one of the non-pretreated group died in the same period. Pretreatment of 8 males with larger doses of pregnandiol (750 gamma daily for 15 days) gave similar results. Removal of the uterus and ovaries prior to adrenalectomy did not alter the resistance of the rats to oestrone, provided sufficient time was allowed for recovery from the first operation.

In a series of six 54-day-old female rats oestradiol proved to be toxic in daily doses of 100 gamma for 10 days and caused a significant decrease in thymus weight—241 mg. compared with 325 mg. for the control animals.

Cholestenone, cholestenone acetate and dehydroandrosterone acetate were each used in 2 mg. doses on six 47-day-old females. Six cholesterol-treated animals served as controls. None of these substances caused thymus involution. Dehydroandrosterone acetate was the only one of the group which had any appreciable toxic action in the doses used. It also had some sex stimulating activity as shown by positive vaginal smears and uterine oestrus.

Progesterone, 100 gamma daily, resulted in death of 4 out of 7 male rats after 7 injections, whereas 7 females of the same age withstood 15 injections. The thymus gland was not altered.

Androstenediol (100 gamma daily) produced no thymus involution and 4 of the 8 rats died in 15 days.

Testosterone in the same dose also gave negative results in 16 animals. Five hundred gamma doses used on 5 rats for 16 days gave an average thymus weight of 258 mg. compared with 317 mg. for the control animals. As the difference was of questionable significance another group of 6 was treated with one mg. doses of

testosterone propionate for 14 days, with marked effect. The thymus decreased from an average of 327 mg. to 160 mg.

We conclude that thymus involution occurs in adrenalectomized rats following the administration of substances other than oestrone. Both oestradiol and testosterone were effective. None of the sexually inert substances caused any thymus change. This suggests a relationship between the sex stimulating property of the sterols and the thymus effect, but the amount necessary to cause thymus involution is many times that required for the physiological effect. The toxicity of the sterols in adrenalectomized rats also appears to run parallel with their physiological activity.

9282

Serum Sodium in Relation to Liver Damage and Hyperthyroidism.*

SVEND PEDERSEN, WALTER G. MADDOCK AND FREDERICK A. COLLER. (Introduced by L. H. Newburgh.)

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The liver of patients with severe hyperthyroidism has shown impairment of function and marked pathological changes.[1] In a search for the cause of thyroid crisis, we were unable to find any definite relation between impairment of liver function, as measured by blood bilirubin and bromsulphalein dye retention, and the degree of postoperative reaction, the latter being of the same nature and in its severe form, true thyroid crisis.[2]

Our attention was then drawn to a publication by Schneider[3] concerning a marked disturbance of serum sodium in relation to liver damage and hyperthyroidism. This investigator has shown experimentally in guinea pigs that daily injections of the thyrotropic hormone from the anterior lobe of the pituitary gland or injections of thyroxine reduce serum and liver sodium below one-half of its normal value, while potassium and chlorides are not significantly

* This investigation was assisted by a grant from the Horace H. Rackham School of Graduate Studies.

1 Weller, C. V., *Ann. Int. Med.*, 1933, **7**, 543.

2 Maddock, W. G., Coller, F. A., and Pedersen, S., *West. J. Surg.*, 1936, **44**, 513.

3 Schneider, E., *Internat. Clin.*, 1934, **2**, 87. Schneider, E., Widmann, E., *Deutsche Z. f. Chir.*, 1933, **241**, 15, 778; *Z. f. d. ges. exp. Med.*, 1933, **90**, 45. Schneider, E., *Klin. Wchnschr.*, 1933, **12**, 1708.

changed. The reduction of serum sodium paralleled the depletion of glycogen in the liver and he stated that thyrogenic liver damage could be followed indirectly by determining sodium in the serum. In a transfer of his study to humans, Schneider found in 6 cases of hyperthyroidism the following values for serum sodium in mg. %: 53.0, 74.0, 134.0, 138.5, 162.0, and 195.0.

When one realizes that sodium, according to McCance,[4] comprises about 94.0% of the total base of the extracellular fluids, except gastric juice and semen, then it is evident that a tremendous decrease in the alkali reserve and great changes of the osmotic pressure of the blood must have taken place in these patients, since there was no compensation of the alkali reserve by a decrease in chlorides. Serum sodium as low as 53.0 mg. % has never been reported, as far as we are aware, in hyperthyroidism or any other disease and is in our opinion incompatible with life.

The purpose of this investigation was to verify Schneider's work on humans, and serum sodium in a group of 10 patients with hyperthyroidism, 9 of whom had evidence of impaired liver function by other tests, is herewith reported.

In this work, serum sodium was determined according to the method of Butler and Tuthill[5] except for minor modifications. Plasma bilirubin was determined by the standard method and figures from 1.0 to 3.0 mg. per liter were considered to be normal. For the bromsulphalein excretion test 5.0 mg. of the dye per kilo of body weight was administered. A retention of 10% or more after 30 minutes was considered to be abnormal.[6]

The data from the 10 cases of hyperthyroidism are presented in Table I. Eight of the 10 had evidence of marked hyperthyroidism, two (9 and 10) dying on the Medical service, two (2 and 3) developing severe thyroid crisis postoperatively during which time one of them died, and one (4) had jaundice preoperatively for which no other cause except toxic hepatitis associated with severe hyperthyroidism could be found.

Concerning the evidence of liver damage from the plasma bilirubin or bromsulphalein dye retention data, 9 of the 10 patients (except No. 10) showed impaired function. The serum sodium, however, during the height of the hyperthyroid reaction, was normal in 7 of the 10 cases and only a little below 300 mg. % in the remain-

[4] McCance, R. A., The Goulstonian Lectures on Medical Problems in Mineral Metabolism. Reprinted from *The Lancet*, March 21, 1936, p. 643, March 28, p. 704, April 4, p. 765, and April 11, p. 823.

[5] Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

[6] Soffer, L. J., *Medicine*, 1935, **14**, 185.

TABLE I.
Serum Sodium in Relation to Liver Damage and Hyperthyroidism.

Patient	Age	Diagnosis	BMR %	Serum Sodium Mg. %	Evidence of Liver Damage: Blood Bilirubin	Evidence of Liver Damage: Bsp.* dye retention	Severity of disease
					mg.	%	
1	39	Exophthalmic goiter	+77	308.5	4	20	General evidence of marked toxicity.
2	36	” ”	+70	310.8	2	60	Considerable toxicity. Died postoperatively in thyroid crisis.
3	17	” ”	+75	308.7	2	30	Considerable toxicity. Developed severe postoperative thyroid crisis.
4	65	Toxic adenoma	+39	291.0	60	100	Jaundice from liver damage associated with hyperthyroidism.
5	39	Exophthalmic goiter	+63	279.0	4	15	Considerable toxicity.
6	23	” ”	+26	304.0	3	15	Moderate toxicity.
7	44	Toxic adenoma	+30	311.6	16	100	” ”
8	28	Exophthalmic goiter	+65	354.6	2	35	Considerable toxicity.
9	59	Toxic adenoma	+31	316.5	8	—	Irrational. Died before operation
10	39	” ”	+58	281.9	3	10	Depressed thyroid type. Died before operation.

* Bromsulphalein.

ing 3. The 2 patients with typical thyroid crisis were among the 7 cases having normal serum sodium.

In this study, determinations of serum sodium did not show impaired liver function in a group of patients known to have impaired function by other tests. The work of Schneider in this regard was, therefore, not confirmed. In addition, Schneider stated that operative treatment could be safely employed in patients with hyperthyroidism in whom the risk from clinical indications alone appeared to be excessive, if the serum sodium was above 100 mg. % and reaching 200 mg. %. This was not used as the indication for operation in this series of cases, and fortunately so, since 3 of these patients (2, 9, and 10) died, 2 failing on conservative treatment to be improved to the point that operation could even be considered.

From this study it is apparent that serum sodium determinations have no value in relation to hyperthyroidism found in a typical North American goiter district. Essentially the same findings have been obtained by Feldmaus[7] from a similar investigation in Poland, the publication of which appeared in the literature after our work had been completed.

9283 P

Effect of Trypsin on the Clotting of the Blood in Hemophilia.

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It is known that trypsin will coagulate blood. Its effect on the blood in hemophilia has been studied using crystalline trypsin obtained through the courtesy of Dr. John Northrop.[1] Blood was drawn from the antecubital vein of patients with hemophilia into an oiled glass syringe, great care being taken to avoid unnecessary trauma, and cautiously run into clean glass test tubes. These were let stand at room temperature and carefully tilted every 5 minutes until clotting occurred. Duplicate observations were averaged. The clots retracted and liquefied more rapidly when large amounts of trypsin were used.

A similar experiment was carried out using placental extract,

[7] Feldmaus, B., *Acta. Med. Scandinav.*, 1936, **88**, 39.

[1] Northrop, J. H., The Harvey Lectures, 1934-5, **30**, 229.

obtained through the courtesy of Dr. R. C. Eley,[2] instead of trypsin. The addition of 0.3 cc., whether boiled or unboiled, to 5 cc. of blood reduced the clotting time from 72 minutes to 5 minutes.

Oxalate was then added to normal and to hemophilic blood in sufficient concentration to prevent clotting over night. The addition of 0.3 cc. of a 5% solution of oxalated commercial trypsin (Fairchild and Foster) to the oxalated bloods was followed by clotting in 10 minutes.

When 5 mg. of heparin per 5 cc. of blood was substituted for oxalate, the addition of commercial trypsin did not cause clotting in either normal or hemophilic blood. Fresh normal human serum acted in a similar manner to trypsin. When 0.2 cc. of serum was added to 5 cc. of oxalated normal and oxalated hemophilic blood both clotted within 4 minutes; whereas, when the same amount of serum was added to 5 cc. samples of heparinized normal and heparinized hemophilic blood, no clotting occurred.

TABLE I.

Hemophilic blood	Crystalline trypsin in 0.7% NaCl. 0.1 cc. = 0.3 mg.	Clotting time
cc.	mg.	min.
5	0	85
5	0.3	30
5	0.6	10
5	1.2	3
5	1.2 (boiled)	90

The addition of 0.1 cc. of fresh normal human serum to 5 cc. of hemophilic blood reduced the clotting time from 70 to 4 minutes, while the addition of the hemophiliac's own fresh serum was without effect in doses of 0.2 cc. but reduced the clotting time from 80 to 25 minutes when 0.4 cc. was used. This effect was wholly destroyed by boiling the sera. This is in accord with the findings of Patek.[3]

The oral administration of the placental extract, chilled and fed with calcium carbonate on an empty stomach, was without effect on the clotting time in 2 cases of hemophilia (aged 26 and 14 years), as was the oral administration of 30 gm. of commercial trypsin daily under similar conditions, and of 12 gm. daily in enteric coated capsules.

The intravenous injection of commercial trypsin solution in a rabbit caused instant death with thrombus formation on the right auricle.

[2] Eley, R. C., Green, A. A., and McKhann, C. F., *J. Pediat.*, 1936, **8**, 135.

[3] Patek, A. J., Jr., and Taylor, F. H. L., *J. Clin. Invest.*, 1937, **16**, 113.

Summary. Trypsin accelerates the coagulation of hemophilic blood *in vitro*. Its action is similar to that of thrombin.

9284

Relation of Urinary Excretion of Estrone to Menstrual Cycle of Normal Woman.

LEONARD D. YERBY. (Introduced by W. R. Lyons.)

From the Division of Anatomy, University of California Medical School.

Although earlier work[1, 2] had failed to demonstrate a greater efficacy of the intravaginal method of assaying estrone, Berger[3] reported that 1/12th of the parenteral unit of estrone could be detected by intravaginal administration. Lyons and Templeton[4] reported that by their intravaginal method 1/200th of a rat unit of estrone could be detected. The latter workers reported estimates of the amount of urinary estrogenic substance excreted daily, at 4 periods in the normal menstrual cycles of 4 nulliparous women.

It seemed desirable to follow the daily excretion of estrogenic substance throughout the cycle, and for this purpose the cooperation of 2 normal nulliparous women was secured. For more than a full cycle these women collected 24-hour samples of urine preserving them with hexylresorcinol (1 part to 20 of urine) and storing them at 0°C. Crude extracts were made of a fraction of each sample according to the method formerly given,[4] and these extracts were administered vaginally to rats by means of a Breed and Brew 0.01 cc. micro-pipette to which was attached a short rubber tube such as is used on a blood-diluting pipette. Care was taken to remove any of the extract adhering to the outside of the pipette before inserting in the vagina.

Of a group of 40 ovariectomized adult rats, 24 were found sufficiently uniform and consistent in their reaction to 1/200th of a rat unit of Progynon B* administered intravaginally, to permit their

[1] Pratt, J. P., and Schmeltzer, M., *Endocrinology*, 1929, **13**, 320.

[2] Powers, H. H., Varley, J. R., and Morrell, J. A., *Endocrinology*, 1929, **13**, 395.

[3] Berger, M., *Klin. Wochnschr.*, 1935, **14**, 1601.

[4] Lyons, W. R., and Templeton, H. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 587.

* The Progynon B (in oil) was supplied to Dr. H. M. Evans by the Schering Corporation of New Jersey. Dilutions were made with sesame oil. It is probable that differences encountered in standardizing estrone in oil and in water by the

use as assay animals. In standardizing the rats as well as in testing, the preparations were administered in 2 0.01 cc. daily doses and the reaction read 24 hours after the second administration.

The minimal effective dose was considered the smallest amount of extract that would produce a vaginal smear showing that definite epithelial growth and cornification had been stimulated in at least 2 standardized rats—lower doses giving negative reactions and higher doses producing more complete cornification.

Case A was studied throughout a 30-day cycle (an average length for this woman). Case B was studied throughout a 21-day cycle, the average length of cycle for this woman being 25 days. Given in the accompanying chart and Table I are the number of intravaginal units excreted daily by Case A, the 24-hour urinary volumes and the minimal effective urinary equivalent. It will be observed that the excretion of estrogenic substance reached two definite peaks during the menstrual cycle. One of these at mid-cycle (ovulation time), and the other, just before menstruation. In the case of the 21-day cycle, the mid-cycle peak occurred on day 9 and the premenstrual peak on day 17. Several other workers[5, 6, 7] have re-

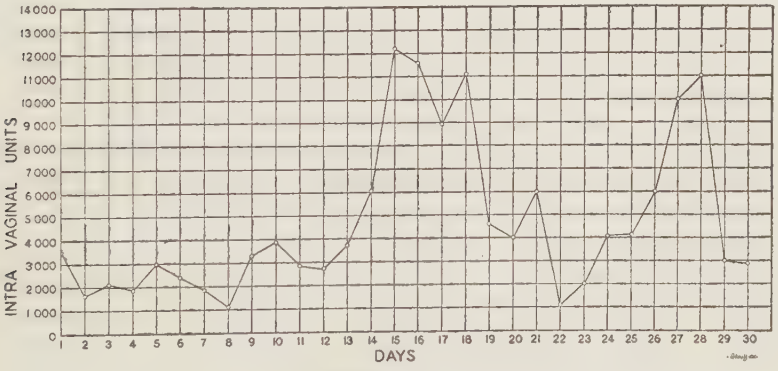

CHART 1.

Graph showing the number of intravaginal units of urinary estrogenic substance excreted daily throughout the menstrual cycle of a normal, nulliparous young woman (Case A).

subcutaneous method will be minimized by the intravaginal method, since rates of absorption and excretion are important variables to be considered in accounting for these differences. No attempt is made to identify the urinary estrogenic substance assayed in this research with any of the various estrones. Those interested in translating the unitages reported herein to one of the other systems should be able to do so through the medium of the Progynon B standardization (see also Schoeller, W., *et al.*, *Klin. Wchnschr.*, 1935, **14**, 826.).

5 Gustavson, R. G., and Greer, D. F., *J. Biol. Chem.*, 1934, **105**, XXXIV.

6 Smith, G. V. S., and Watkins, O., *Am. J. Physiol.*, 1935, **112**, 340.

7 Pedersen-Bjergaard, K., *Zentralblat. f. Gynäk.*, 1936, **60**, 372.

ported finding 2 peaks of estrone excretion in woman. Fluhmann[8] reported that the blood estrone reached a peak at the 14th day, gradually declining until the 27th day.

TABLE I.

Day of Cycle	Volume of Urine	Min. Effective Urinary Equiv.	Units per 24 hr.	Day of Cycle	Volume of Urine	Min. Effective Urinary Equiv.	Units per 24 hr.
	cc.	cc.			cc.	cc.	
1*	1050	.3	3500	16	585	.05	11700
2*	670	.4	1675	17	880	.1	8800
3*	680	.3	2266	18	1120	.1	11200
4*	1290	.7	1843	19	1400	.3	4666
5*	1185	.4	2962	20	1220	.3	4066
6	1190	.5	2380	21	1800	.3	6000
7	930	.5	1860	22	1250	1.0	1250
8	640	.5	1280	23	600	.3	2000
9	690	.2	3450	24	1230	.3	4100
10	1140	.3	3800	25	1300	.3	4333
11	1400	.5	2800	26	1800	.3	6000
12	820	.3	2733	27	1600	.16	10000
13	750	.2	3750	28	1100	.1	11000
14	610	.1	6100	29	1200	.4	3000
15	1230	.1	12300	30	870	.3	2900
				1	Menstruated.		

* Menstruating.

The mid-cycle peak is probably due—as many investigators have suggested—to an increased production of estrone by the ripe Graafian follicle; but the subsequent drop and premenstrual rise will probably only be understood when a sensitive enough method will allow for the estimation of estrone and progesterone in the same sample. The figures submitted in this report represent the daily excretion by normal women of estrogenic substance detectable in urine by a given method; they do not represent the actual amount of estrone produced by the ovary.

[8] Fluhmann, C. R., *Endocrinology*, 1934, **18**, 705.

9285

Comparative Toxicity of Sixteen Specimens of *Crotalus* Venom.

David I. Macht.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Sixteen specimens of *Crotalus* venom were obtained through the courtesy of Dr. L. M. Klauber, Curator of the Natural History Museum of Balboa Park, San Diego, California, and all of these were biologically assayed for their toxicity. One specimen contained a mixture of venoms from several species of *Crotalus;* two were taken from the *Crotalus cinereous;* and the remainder were derived from 13 different varieties of the same species. The venoms were obtained by a uniform method under identically the same conditions in each case. They were all centrifuged and then dried in a vacuum at a relatively low temperature because *Crotalus* venom, when exposed to temperatures as high as 100°F., deteriorates. Since the age of the different specimens varied, the writer had an excellent opportunity to make not only a comparative study of their toxicity but also an inquiry into the effects of the lapse of time on the keeping qualities of rattlesnake venom. The toxicity of the respective venoms for animals and plants was therefore investigated. In zoöpharmacological experiments, the writer determined the minimal lethal dose of these specimens by intraperitoneal injection in white mice and, in phytopharmacological experiments, studied the effect of solutions of *Crotalus* venom in equal parts of Shive saline and distilled water (concentrations of 1:20,000 and 1:10,000) on the growth of seedlings of *Lupinus albus* with an initial root length of from 35 to 50 mm. The phytopharmacological test has been described elsewhere.[1] In the present investigation the increment in growth of the seedling roots for 24 hours in the dark at 15°C. was taken as a criterion. Table I exhibits the data obtained regarding all the specimens of *Crotalus* venom investigated.

The dosages for mice were expressed as the average lethal dose for a mouse weighing 22 gm. It will be noted that there was a wide divergence in toxicity of the venoms for both animals and plants. The minimal lethal dose of the most toxic specimen for mice was 0.045 mg. while that of the least toxic was 0.30 mg., or nearly 6.7 times as much. This difference in toxicity obviously bears no relation to the age of the specimens, for some of those recently col-

[1] Macht and Livingston, *J. Gen. Physiol.*, 1922, **4**, 573.

TABLE I.
Comparative Toxicity of Specimens of *Crotalus* Venom.

Lot No.	Species of *Crotalus* from which Venom was Obtained	Date Collected	Minimal Lethal Dose for Mice	Phytotoxic Index of Growth of *Lupinus albus* Seedlings at 15°C. in	
				1:20,000 Venom Sol.	1:10,000 Venom Sol.
			mg.	%	%
586	Mixed *Crotalus* venoms	6- 3-35	.07	66	50
441	*Crotalus cerastes*	6-10-32	.06	87	64
540	*Crotalus mitchellii mitchellii*	8-27-33	.045	69	55
422	*Crotalus viridis viridis*	5-16-32	.045	66	59
465	*Crotalus viridis abyssus*	10-13-32	.10	59	48
489	*Crotalus cinereus*	5-21-33	.12	77	66
551	*Crotalus viridis lutosus*	10-29-33	.14	83	77
545	*Crotalus viridis oreganus*	9-10-33	.14	87	81
587	*Crotalus scutulatus*	6-10-35	.24	91	80
530	*Crotalus molossus molossus*	8-13-33	.06	87	76
518	*Crotalus mitchellii pyrrhus*	7- 9-33	.075	83	73
496	*Crotalus ruber*	7- 2-35	.11	86	79
588	*Crotalus cinereus*	6-10-35	.30	94	93
562	*Crotalus mitchellii stephensi*	4- 8-34	.12	85	78
584	*Crotalus horridus*	6- 3-35	.11	85	80
517	*Crotalus lucasensis*	7- 6-33	.12	81	80

lected were quite weak while some of the oldest were among the most potent venoms examined. Of special interest is the marked difference in toxicity of specimens 489 and 588, taken from the *Crotalus cinereus* in 1933 and 1935, respectively. The reason for this difference in toxicity of 2 specimens obtained with the same technique under parallel conditions is problematical but a number of factors, such as age, health, nutrition, light, temperature and barometric pressure, may play a rôle in this connection.[2, 3] The present research was carried on for a period of 5 months. The lethal dosage of the first 2 venoms examined, specimens 586 and 441, was discovered to be the same at the expiration of the 5-month interval as it had been at the beginning, a finding which indicated that the potency of specimens kept at a temperature of from 12 to 17°C. in the dark remained unchanged. This is not true, however, of saline solutions of *Crotalus* venom, as these rapidly deteriorate not only when exposed to sunlight but also when kept even at room temperature.

The toxicity of the different specimens for plants also varied and in general ran parallel to that which they respectively exerted on animals. However, quantitative variations were noted when their toxicity for plants and animals, respectively, was compared, differences which were to be expected since snake venoms are not single entities but are made up of several constituents, some of which may be more effective for plants as others are for animals. The study outlined above is impressive particularly in respect to the enormous variation in potency of snake venoms obtained by uniform method under the same conditions.

Summary. Sixteen specimens of dry *Crotalus* venoms from 14 different species of rattlesnake were tested biologically for their toxicity on mice and *Lupinus albus* seedlings. The different specimens varied enormously with regard to their toxicity. The dried scales of *Crotalus* venom kept in the dark and in cool containers retained their potency quite well, in some cases, even for several years.

[2] Noguchi, Snake Venoms. Carnegie Institution of Washington, 1909.

[3] Macht, *Am. J. Med. Sciences*, 1935, **189**, 520.

9286

Intravenous Pentobarbital Anesthesia in Rabbits.

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Sixty mg./kg. of ethyl-l-methyl-butyl barbituric acid (pentobarbital, nembutal) one of the shorter-acting barbiturates is fatal to the majority of 10 rabbits when administered intraperitoneally. Forty mg./kg. (66.6%) of the lethal intraperitoneal dose produces surgical anesthesia for 40 to 50 minutes in a majority of instances within 20 to 40 minutes. Because of its safety this is the usual mode of administration of choice in most laboratory demonstrations and experiments. While the intravenous mode has the advantage of being virtually immediate in the induction of surgical anesthesia in rabbits and other small laboratory animals it is generally regarded as being too dangerous for routine laboratory employment. To this opinion we have never subscribed. Accordingly, we undertook to try to solve the supposed problem of danger incident to the intravenous employment of pentobarbital and thereby take advantage of its rapidity of action. In this work we were able to compile a large series of cases by employing pentobarbital intravenously over a period of 6 years in weekly operations on female rabbits as a part of the obstetrical and gynecological services of the Freedmen's Hospital in tests for early pregnancy (Schneider-Friedman Modification of the Aschheim-Zondek Test). This series supplemented by another large series in which this agency among others has been used in experimental pharmacology in our laboratory makes a total of 2,500 cases in which we have employed pentobarbital intravenously in surgical anesthesia in rabbits.

Correct dosage is a matter of prime importance. After numerous trials varying the single dose symptomatically the average optimal figure was found to be 20 mg./kg. We usually weigh out the barbiturate as the sodium salt because of its ready solubility. When the acid is weighed out and rendered soluble by the addition of sodium hydroxide allowance is made for the differences in molecular weights (110 mg. salt = 100 mg. acid). A 5% aqueous solution is usually employed.

The animal is either held down on its side by an assistant or strapped to a rabbit board on its back. The injection is made into the marginal ear vein at a slow rate (*circ.* 1 cc. per 30 sec.). As

soon as muscular relaxation sets in and the respiration slows, the rate of injection is reduced, the operator making brief pauses at intervals to gauge the latency and thereby avoiding the facile overdose. To measure grossly the degree of narcosis the tip of the ear or tail is pinched. Failure to resist by an outcry is determinative. The operative field can then be prepared and the incision made. Usually effective anesthesia has been obtained by this time (2 to 5 minutes). Occasionally a very small supplementary dose may be indicated just before making the incision.

The intravenous dose of pentobarbital necessary to produce good surgical anesthesia in rabbits varies in individual cases from 17.5 to 25 mg./kg. In our experience the optimal dose is 20 mg./kg. For the guidance of those who would want to use this procedure we advise injecting the first 15 mg./kg. at the rate of 1 cc. per 30 sec., then stopping for 10 sec., and following with 5 mg./kg. in 2, 3, or more squirts at intervals of 5 to 10 sec. p.r.n. The result in 98% of the cases will be satisfactory. If, on account of a too long latency as may occasionally happen an overdose has been given, an intravenous dose of picrotoxin invariably furnishes adequate protection. The optimal antidotal dose of picrotoxin is 1 mg. picrotoxin per 9 mg. nembutal. (Maloney, Fitch, and Tatum[1]). This dosage of picrotoxin is varied in accordance with the symptomatic response of the individual animal. Pentobarbital used as here described, is rapid, safe and satisfactory

9287 P

Production of Experimental Hypospadias in the Female Rat.

R. R. GREENE. (Introduced by A. C. Ivy.)

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It has been reported that the injection of large amounts of estrogenic substances into the pregnant rat during the last few days of her pregnancy caused an "hypospadias" in the female offspring.[1, 2] This same abnormality resulted when the mother rat was injected with large doses during the first 4 days of lactation.[3, 4] It was also

[1] Maloney, Fitch and Tatum, *J. Pharm. and Exp. Therap.*, 1931, **41**, 465.

[1] Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 131.

[2] Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 303.

[3] Hain, A. M., *Edin. Med. J.*, 1935, **42**, 101.

[4] Hain, A. M., *Quart. J. Exp. Physiol.*, 1936, **26**, 29.

produced by direct injection of the female newborn.[4]

In the normal adult female rat the urethra opens at the apex of the clitorine prominence. In these abnormal animals the clitoris is widely split and the urethral meatus is immediately cephalad to the vaginal orifice. The lesion apparently involves the integrity of the urinary sphincter, as incontinence is usually present.

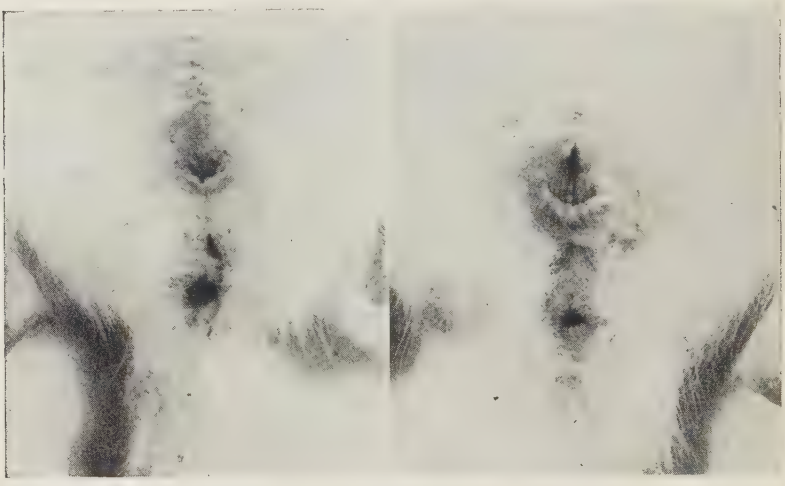

FIG. 1.

Normal Rat. Abnormal Rat.

These findings have been confirmed during the course of a study on lactation. No effect has been noted in the male offspring. An attempt to produce the lesion in the female newborn by direct injection after the seventh day of life has been unsuccessful.

The animals treated to date fall into 3 groups. The first group consisted of 9 litters in which the mothers were injected with estrone in the postpartum period only. The mothers of 7 litters were injected with a total of 0.25 mg. each during the first 5 postpartum days. None of the female offspring had the abnormality. Two mothers were injected with 2.0 mg. during the postpartum period. One of these animals was given this dosage during the course of the first 5 days. None of her female offspring had the lesion. The other animal was given this dosage during the first 2 postpartum days. Three of her 4 female offspring had this lesion.

In the second group of animals treatment was given antepartum only. The abnormality was present in the 4 female offspring of one female that had 1.0 mg. during the 24 hours preceding delivery, and in the 5 female offspring of one animal that was given 2.0 mg. in the 48 hours preceding delivery.

TABLE I (Group 3).

Litter No.	Antepartum Dosage mg.	Days of Antepartum Treatment	Postpartum Dosage mg.	Days of Postpartum Treatment	No. of Females	No. with Lesion	No. without Lesion	% of Hypospadias
63	0.2	1	1.0	5	3	3	0	100
64	0.2	1	1.0	5	3	3	0	100
71	0.2	1	1.0	5	4	4	0	100
83	0.2	1	1.0	5	1	1	0	100
65	0.3	2	1.0	5	6	6	0	100
76	0.3	2	1.0	5	4	0	4	0
68	0.4	2	1.0	5	6	6	0	100
84	0.4	1	2.0	5	4	0	4	0
72	0.5	3	1.0	5	1	1	0	100
75	0.9	5	1.0	5	2	1	1	50

In the third group the 10 mothers received treatment both ante and postpartum. The antepartum treatment varied. All except one animal received 0.1 mg. 2 times daily for the first 5 postpartum days. This one animal received twice this postpartum dosage. Of the 34 female offspring in these litters, the abnormality was present in 25.

Hypospadias has been produced so far in 37 female rats. Twenty-eight untreated litters have been observed specifically for this lesion. It has not been observed in any of the 128 female offspring of this group.

Inasmuch as the urethra of the newborn female rat opens immediately in front of the rectum and the clitorine prominence displays a grossly visible cleft, it is suggested that this abnormality represents an hypotrophic rather than a hypertrophic developmental defect.

9288 P

Action of Morphine Sulphate on Intestinal Motility and its Modification by Atropine Sulphate.

MUNIR A. KANAN. (Introduced by George H. Miller.)

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The experiments were conducted on unanesthetized female dogs with weights ranging between 12-18 kg. Two Thiery Vella loops of adjacent parts of the ileum were prepared, which enabled us to record, in the same dog simultaneously, changes in tone, rhythmic and peristaltic movements in one loop by the balloon method,[1] and the actual propulsive activity in the second loop. For the latter purpose the method of Quigley, Highstone and Ivy was followed; the bolus used was made of rubber crepe of fine quality soaked in liquid paraffin.[2]

Morphine sulphate in doses of 1 mg. per kilo injected subcutaneously causes an increase in tone, diminishes segmentary movements and abolishes peristaltic waves. This confirms the results of the experiments of Plant and Miller.[3]

The propulsive activity after this dose of morphine is at first in-

[1] Plant, O. H., *J. Pharm. and Exp. Therap.*, 1921, **16**, 312.

[2] Quigly, J. P., Highstone, W. H., and Ivy, A. C., *Am. J. Physiol.*, 1934, **108**, 151.

[3] Plant, O. H., and Miller, G. H., *J. Pharm. and Exp. Therap.*, 1926, **27**, 361.

creased and this initial increase in the speed of propulsion is later followed by a marked decrease. While the normal time for a bolus to pass through an intestinal loop of 9-10 cm. in length varied between 6-12 minutes, the same distance was covered within 20-40 seconds after morphine. The period of increased speed of propulsion started within 1-2 minutes after the morphine-injection and lasted for an average of 20-40 minutes. After that the speed of propulsion changed abruptly, becoming much slower than normal; the latter condition lasting for about an hour.

While these results agree in general with those of Quigley, Highstone and Ivy[4] they differ in details. In our experiments the duration of the period of increased propulsive activity was longer and the maximal speed of propulsion was greater.

According to Plant and Miller[3] atropine sulphate in doses sufficient to paralyze the vagus (0.2 mg. per kilo subcutaneously) has no apparent influence on the effect of morphine sulphate on tone, segmentary and peristaltic movements of an intestinal loop of the ileum in a Thiery Vella dog (recorded with the balloon method).

Gruber, Greene, Drayer, and Crawford, however, found that the increased tone caused by morphine could be antagonized completely by a following injection of atropine. They gave in one of their 2 experiments one mg. and in the other 1.4 mg. of morphine sulphate per kilo intravenously, followed by 0.5 mg. and 0.35 mg. respectively of atropine sulphate intravenously.[5]

In dogs with 2 Thiery Vella loops atropine in a dose of 0.2 mg. per kilo subcutaneously, which was found to abolish the inhibitory influence of the vagus on the heart, invariably abolished the increased rate of propulsive activity produced by morphine. The intestinal effect always coincided with the increasing effect of atropine on the heart. Atropine given before morphine prevented the increase in propulsive activity which invariably followed morphine alone.

Our results,* using the same dose and method of administration as Plant and Miller, confirm their findings in that atropine does not have any apparent influence upon tone, segmentary and peristaltic movements altered by morphine. Larger doses of atropine, however, given subcutaneously and the same or larger doses given in-

[4] Quigley, J. P., Highstone, W. H., and Ivy, A. C., *J. Pharm. and Exp. Therap.*, 1934, **51**, 308.

[5] Gruber, C. M., Greene, W. W., Drayer, C. S., and Crawford, W. M., *J. Pharm. and Exp. Therap.*, 1930, **38**, 389.

* These experiments were made in collaboration with Professor Harald G. O. Holck on dogs with one Thiery Vella loop.

travenously antagonized the effect of morphine in a way similar to the results of Gruber, *et al.*

9289

Progestin and Estrin of Nineteen Placentas from Normal and Toxemic Cases.*

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The purpose of this study was to determine whether placentas from toxemic pregnancy differed significantly from those of nontoxemic pregnancy in content of progestin. Estrin was assayed coincidentally.

Within 48 hours of delivery the placentas, having been kept in the refrigerator, were cleaned of all membranes, drained and blotted, ground finely in a meat grinder, measured and vigorously shaken in 2 volumes of 95% ethyl alcohol, in which they remained until extraction. The methods of Allen[1, 2] were employed for extraction and of Allen and Meyer[3] for the separation of the estrogenic from the progestational fraction. For performing assays on the progestational fraction, which was taken up in olive oil, the Corner-Allen technique[4] was followed. In 6 instances (Nos. 1, 2, 3, 4, 8 and 9) 1/25th of the progestational fraction was removed and tested for estrogenic activity on 2 rats (Allen-Doisy method). Negative results were obtained, showing that these fractions contained definitely less than 50 r.u. of estrogenic substance. The 33% alcoholic estrogenic fractions were assayed directly, dilution, when necessary, being made with 33% ethyl alcohol. Clinical and biological data are tabulated in Table I. Table II summarizes the averages of the biological findings.

Only cases 4, 5, 12 and 18 may be regarded as truly normal pregnancies, the premature deliveries in the other "normals" being evidence of some upset in otherwise clinically normal pregnancies. Presumably the 3 patients whose membranes ruptured spontaneously were in other respects normally pregnant.

* The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy.

[1] Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 174.

[2] Allen, W. M., *J. Biol. Chem.*, 1932, **98**, 591.

[3] Allen, W. M., and Meyer, R. K., *Am. J. Physiol.*, 1933, **106**, 55.

[4] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

TABLE I.
Clinical and Biological Data on Nineteen Placentas.

Patient No.	Diagnosis	Age	Parity	Week of Pregnancy	Type of Delivery	Volume of Placenta	Progestin*	Estrin	
						cc.		r.u.	r.u./cc.
1	Toxemia without convulsions	26	0	37	Spontaneous	180	0	380	2.10
2	" " "	34	3	31	Induced	160	++	230	1.43
3	"Normal"	20	0	32	Spontaneous	230	++	1130	4.91
4	Diabetes. Normal	30	1	38	Cesarean	400	++†	1500	3.75
5	Normal	25	0	Term	Spontaneous	300	0	1320	4.40
6	Toxemia without convulsions	29	2	36	Cesarean	300	+	1500	5.00
7	"Normal." Spontaneous rupture of membranes	35	2	35	Spontaneous	250	+	780	3.12
8	Diabetes. Mild toxemia	24	0	37	Cesarean	300	++	1160	3.86
9	Diabetes. Toxemia without convulsions	27	1	34	"	450	+++	1920	4.30
10	Toxemia without convulsions	28	6	36	Induced	285	+	610	2.40
11	Premature toxic separation	26	0	31	Spontaneous	75	0	390	5.20
12	Normal	34	0	Term	"	300	+++	1500	5.00
13	Premature toxic separation	26	0	36	Cesarean	190	++	116	0.61
14	Toxemia without convulsions	30	1	31	"	150	++++‡	390	2.60
15	"Normal"	20	0	32	Spontaneous	215	+++	1160	5.39
16	"Normal." Spontaneous rupture of membranes	27	1	32	"	170	+++±	580	3.41
17	"Normal"	21	1	29	"	175	++++	390	2.20
18	"	33	1	37	"	340	+++±	1170	3.40
19	"Normal." Spontaneous rupture of membranes	20	0	34	"	250	+++±	1670	6.60

* Willard M. Allen very kindly checked the readings on Nos. 1 13 and 15. Gregory Pincus very kindly checked all the readings
† With 3/5th of progestin fraction. ‡ This patient received 40 mg. progesterone during the 48 hours before delivery.

TABLE II.
Averages of Placental Progestin and Estrin of 9 Cases of Toxemia and 10 "Normals."

Progestin	*Toxemia*	*"Normal"*
0 to +	4	2
++ to ++++	5§	8
Estrin		
Total	941	1132
r.u./cc. wet vol.	3.2	4.3
Time of delivery		
Weeks early	5.7	5.0

§ One of these received 40 mg. of progesterone during the 48 hours before delivery.

Ehrhardt and Fischer-Wasels[5] found positive results for placental progestin in cases of eclampsia and pre-eclampsia but could draw, from quantitative comparisons, no conclusions as to possible etiology.

From the data presented in Table I it must be concluded that an absolute lack of progestin is not associated with either spontaneous delivery or late pregnancy toxemia. In neither of these situations, however, has the possibility of a relative deficiency of progestin been ruled out. The average figures in Table II indicate a somewhat lower content of progestin in the placentas from cases of toxemia, but the results may be considered no more than suggestive, considering the fact that the whole of each extract was tested on a single rabbit and that the method of extraction and assay may at best be considered only roughly quantitative.

The average figures for placental estrin show a tendency for low values in cases of toxemia, thereby confirming our previous findings.[6]

Conclusion. The placentas of late pregnancy toxemia cannot be shown by the methods available to differ significantly in content of progestin from those of non-toxemic pregnancy.

[5] Ehrhardt, K., and Fischer-Wasels, H., *Monatschr. f. Geburtsh. v. Gynäk.*, 1936, **102**, 80.

[6] Smith, G. V., and Smith, O. W., *Am. J. Obstet. and Gynec.*, 1937, **33**, 365.

9290 P

Precipitin Tests with Glycogen from various Species of Animals.*

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Recent studies[1] indicate that polysaccharides from various parasitic helminths are immunologically active, and that their chemical properties are similar to ordinary preparations of glycogen. The present study was undertaken to ascertain whether precipitins could be produced against glycogen from livers of the guinea pig, chicken and frog, and from fresh water clams.

Preparations of glycogen were obtained as follows: finely ground tissue was suspended in 3 volumes of hot distilled water and kept in a boiling-water bath for 30 minutes. The solution, after the water-insoluble material had been discarded, was acidified to approximately pH 5, and left for several hours at room-temperature in 2 volumes of 95% ethyl alcohol. The precipitate which formed was resuspended in 1.0% acetic acid, and after 0.2 volume of 95% alcohol had been added, was chilled for several hours in the refrigerator. All insoluble material was then discarded, and the soluble glycogen precipitated with one volume of alcohol and rapidly dried with absolute alcohol and ether. Such preparations were essentially free of nitrogen and gave no protein reactions.

Rabbits were immunized by 10 intraäbdominal injections of 0.1 gm. of finely ground fresh material of one of the following: guinea pig, chicken or frog livers or fresh water clams. The "ring" method of precipitin-test was used. Serums were diluted 1:2 with 0.9% NaCl and antigens tested in a series of dilutions from 1:100 to 1:51,200.

The rabbit antiserums did not react with glycogen from guinea pig, chicken or frog livers, but anticlam serums did contain precipitin against the glycogen from fresh water clams (titre as dilution of antigen, 1:12,800). There were no cross-reactions between clam-antigens and antigens from various parasitic helminths. Liver-glycogen, therefore, appears to be either immunologically inactive or else the glycogens present in the 4 vertebrates studied (guinea pig, chicken, frog and rabbit) are antigenically identical, whereas glycogens obtained from clams and helminths are immunologically active and specific.

* Aided in part by the Logan Fund of the University of Chicago and in part by a grant from the Rockefeller Foundation.

[1] Campbell, D. H., *J. Infect. Dis.*, 1936, **59**, 266, and *J. Parasitol.*, (in press).

These results are in part contradictory to those obtained by Ikeda,[2] who found that glycogen from mammalian tissues induced organ-specific antibodies when injected into rabbits.

9291 P

Rôle of Thyroid in Increased Protein Metabolism of Phlorhizin Diabetes.*

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The mechanism of phlorhizin diabetes has been the subject of much investigation and controversy. Two distinct views exist, one postulating that the action of phlorhizin is exclusively renal[1, 2] and the other that some primary extrarenal factor also is involved.[3, 4] These views arise from the fact that although no apparent effect can be observed when phlorhizin is administered to dogs without renal function,[2] a definite hyperglycemia,[3] and abnormalities in the glucose tolerance curve[4] may occur in such animals if the phlorhizination is completed before the kidneys are incapacitated.

The observations of Dann, Chambers and Lusk[5] indicate the involvement of extrarenal factors in the genesis of phlorhizin diabetes. They demonstrated that the administration of phlorhizin to the thyroidectomized dog does not result in the increased basal metabolic rate and the increased nitrogen excretion which occur in the intact phlorhizinized dog. During the course of a series of studies on nitrogen metabolism, we had occasion to make some observations which confirm and extend those of Dann, Chambers, and Lusk.

Three groups of animals were studied. The first group consisted of normal dogs which had been fasted for 3 to 4 days; the second, of normal dogs which had received a daily subcutaneous injection

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2 Ikeda, G., *Jap. J. Exp. Med. Sci. Trans.*, 1932, **7**, 231.

* Aided by the David May Fund.

1 Minkowski, O., *Arch. f. exp. Path. u. Pharm.*, 1892, **31**, 85.

2 Deuel, H. J., Jr., Wilson, H. E. C., and Milhorat, A. T., *J. Biol. Chem.*, 1927, **74**, 265.

3 Underhill, F. P., *J. Biol. Chem.*, 1912, **13**, 15.

4 Goldstein, L. A., Tatelbaum, A. J., Ehre, S., and Murlin, J. R., *Am. J. Physiol.*, 1932, **101**, 166.

5 Dann, M., Chambers, W. H., and Lusk, G., *J. Biol. Chem.*, 1931, **94**, 511.

of a suspension of phlorhizin in oil for 3 to 4 days, during which time all food was withheld; the third, of dogs subjected to a complete thyroidectomy and subsequently phlorhizinized.

On the fourth day of fasting and of phlorhizination, a bilateral nephrectomy was performed under sodium amytal anesthesia which was maintained throughout the experimental period. Immediately after the completion of this operation, an arterial blood sample was drawn from an exposed femoral artery for determination of non-protein-nitrogen by a micro-Kjeldahl method (digestion according to the Koch-McMeekin and distillation by the Bock-Benedict method), sugar by the Somogyi modification of the Shaffer-Hartmann method and total solids by drying a weighed quantity of blood to constant weight. Arterial blood samples were drawn again at 2- to 4-hourly intervals for 12 hours. In a number of instances, the experimental period was extended an additional 12 hours in order to note any late changes in the accumulation of blood non-protein-nitrogen.

During the early stages of these studies, all non-protein-nitrogen and sugar values of the blood were corrected for changes in the water content, but this was subsequently abandoned because of the negligible changes observed.

In another communication[6] we have presented evidence to show that the rate of non-protein-nitrogen accumulation in the blood of nephrectomized dogs is as accurate a gauge of protein metabolism as is the determination of urinary nitrogen excretion. Accordingly, the data summarized in Table I reveal that even in the absence of kidneys, the protein metabolism of the phlorhizinized dog is about 30% higher than that of the normal dog. This is true even if the experimental period is extended over 24 hours instead of 12 as herein reported. Thus, it is obvious that the removal of the kidneys does not result in a complete cessation of phlorhizin diabetes. However, when phlorhizin is administered to the previously thyroidectomized dog, the rate of protein metabolism is the same or slightly lower than that of the normal dog after the kidneys are removed. This indicates that the process of phlorhizination probably results in stimulation of the thyroid gland which in turn is responsible for part of the increased protein metabolism observed in phlorhizin diabetes.

In spite of the fact that the rate of protein metabolism is greater in the normal phlorhizinized-nephrectomized dogs than in the previously thyroidectomized, phlorhizinized-nephrectomized dogs, the

[6] Mirsky, I. A., Heiman, J. D., and Swadesh, S., (in press).

TABLE I.

The Blood Non-Protein Nitrogen of Nephrectomized Normal, Phlorhizinized and Thyroidectomized-Phlorhizinized Dogs.

Experiments	No. of Exp.	Blood Sugar							Blood Non-Protein Nitrogen						
		0	4 hr.		8 hr.		12 hr.		0	4 hr.		8 hr.		12 hr.	
		Actual	Actual	Increase	Actual	Increase	Actual	Increase	Actual	Actual	Increase	Actual	Increase	Actual	Increase
		mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%
Normal Controls	5	66	66	0	66	0	70	4	29.7	40.4	10.7	52.3	22.6	66.1	36.4
Phlorhizinized Controls	5	50	106	56	97	47	104	54	45.6	59.2	13.6	74.7	29.1	92.8	47.2
Thyroidectomized-Phlorhizinized	3	31	75	44	95	64	85	84	31.5	43.3	11.5	53.8	20.0	62.5	31.0

blood sugar levels are approximately the same. Thus, both sets of animals have a low initial blood sugar level before nephrectomy, and rise to a level somewhat higher than that observed in normal animals after nephrectomy. This post-nephrectomy blood sugar level is maintained constant over the period of time studied. Hence, it is probable that the blood sugar *per se* is not the factor responsible for the stimulation of the thyroid gland.

Summary and Conclusions. The administration of phlorhizin results in a stimulation of the thyroid gland which in turn is responsible for part of the increased protein metabolism observed in phlorhizin diabetes. Since this increased protein metabolism of the phlorhizinized animal continues even after the removal of the kidneys, but does not occur when phlorhizin is administered to previously nephrectomized animals,[2] it is probable that this drug exerts some specific effect on the kidney, which is in turn responsible for the thyroid stimulation. Our observations do not offer evidence for the possibility that the blood sugar level is the responsible factor in the increased activity of the thyroid but suggest some primary renal factor.

9292 P

Deleterious Effects of Insulin Shock.

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The effects of a hypoglycemia of long duration on the various tissues and the organism as a whole are of fundamental importance. On the practical side the possibility of the use of excessive doses of the new slow acting protamine insulin by untrained individuals, because of the lack of immediate results or the accidental administration of the concentrated precipitate from a vial which has not been agitated, make essential a knowledge of the effects of hypoglycemia due to insulin shock.

In experiments on dogs we have found that when a state of insulin shock is maintained for 24 hours or longer with protamine zinc insulin it is apparently impossible to resuscitate the animal and death always ensues. Often this result follows when the period is shorter. A typical protocol follows: A male dog weighing 29 kg., last fed the day before the experiment started, was given 40 units (1 cc.)

of protamine zinc insulin,* and 23 hours later 20 units more. Five hundred cc. of fluid were given by stomach tube at 23, 30, 38, 50, 57, and 71 hours. With the exception of the first dose this was 10% sucrose and at 38 and 57 hours 5 gm. of sodium chloride were also administered. The blood sugar concentration was 61 mg. % at 0 hours and 22 at 6.5, 36 at 23, 28 at 25, 22 at 30, 28 at 38, 73 at 47, 52 at 50, 67 at 56, 41 at 71, and 67 mg. % at 73 hours respectively. At this time the dog died, apparently of circulatory failure. The animal had his first convulsion 7 hours after the first dose of insulin and became unconscious within 13 hours. He never came out of this coma which after 40 hours could have had no relation to the blood sugar concentration at the moment. In other experiments milk or milk and sugar has been given with the same result. In many of our experiments death has resulted from hypoglycemia before sufficient sugar was administered. So far we have had seven animals in which death ensued long after the blood sugar level had been returned to normal. Fat and well nourished dogs appear to suffer less from the hypoglycemia than thin ones.

Experiments are being carried out in an attempt to determine the relation of the undesirable effects of insulin shock to the hypoglycemia itself and to the secondary effects on the circulation and other body mechanisms. An important point is the length of the shocking period which is necessary to produce such damage that recovery does not occur. Grossly the brains do not appear particularly abnormal, but it seems possible that damage to the brain, analogous to that due to anoxemia, may explain the untoward effects of hypoglycemia. Insulin shock is being purposely used in schizophrenia therapy[1, 2] and Steinfeld[3] has reported that circulatory collapse or epileptiform convulsions are serious side reactions which may occur even when the blood sugar has again been raised to a normal level. We have seen this happen in our experiments and hypoglycemia is obviously not the immediate cause of the death of our animals.

* We are indebted to Eli Lilly and Company of Indianapolis for the supply of protamine zinc insulin used in this study.

[1] Sakel, M., *Neue Behandling der Schizophrenie,* Vienna, M. Perles, 1935.

[2] Dussik, K. T., and Sakel, M., *Z. f. d. ges. Neurol. u. Psychiat.,* 1936, **155**, 351.

[3] Steinfeld, J., *J. Am. Med. Assn.,* 1937, **108**, 91.

9293

Prolactin-Like Reaction Produced by Hypophyses of Various Vertebrates.*

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Lyons and Page,[1] by intradermal injections of very small amounts of prolactin-containing substances over the crop gland of the pigeon, were able to detect amounts of prolactin 100 times smaller than can be detected by intramuscular injection (confirmed by Bates and Riddle[2]). McQueen Williams,[3] Burrows and Byerly,[4] and Reece and Turner[5] implanted pituitary bodies in the same region and were able to determine the amount of prolactin contained in them by estimating the amount of local proliferation of the crop wall.

The hypophyses of mammals, birds, reptiles, amphibians and fish have been tested by us for the presence of prolactin by the same method. The reactions of the crop walls were graded with plus signs, in a way similar to that of Burrows and Byerly. The results reported in Table I show that positive reactions have been obtained with hypophyses of mammals, birds, reptiles, amphibians and fish.

The specificity of the growth response of the crop gland may be doubted, however, since positive reactions have been observed after implantations with livers of pigeon, turtle, catfish and sunfish, also with brains of sunfish and catfish. In these cases, as after implants of pituitary bodies of amphibians, reptiles and fish, undulated thickenings could be observed, but few or no fat granules were visible in sections of these thickenings following staining with Scharlach R. Such granules are numerous in sections of thickenings produced by treating immature pigeons with small doses of prolactin.†

Conclusion. A prolactin-like reaction is produced by implantations of hypophyses of animals representative of the main classes of

* These studies have been aided by a grant from the Josiah Macy, Jr., Foundation.

1 Lyons, W. R., and Page, E., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1049.

2 Bates, R. W., and Riddle, O., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 847.

3 McQueen Williams, M., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 406.

4 Burrows, W. H., and Byerly, T. C., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 841, 844.

5 Reece, R. P., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 402; 1936, **35**, 60, 367.

† Product of Parke, Davis & Co. We are indebted to Dr. Oliver Kamm for this material.

TABLE I.
Local Prolactin Reactions Produced by Pituitary Bodies of Various Species of Vertebrates.

	No. of pit. implanted	Reaction (No. of pluses)	No. of pit. implanted	Reaction (No. of pluses)	No. of pit. implanted	Reaction (No. of pluses)
Birds Mammals	*Rabbits*		*Mice*		*Rats*	
	2 ♂	4	8 ♂ ♀	3	4 ♂ ♀	2
			20 ♂	4	2 ♂	2
			20 ♀	1-2		
	Chickens ♂		*Chickens* ♀		*Pigeons*	
	3	4	1	?	5 ♂ ♀	3-4
	1	4	2	2		
	1	3	2	3		
	2	3	2 ♂ ♀	4		
	2	4				
Reptiles	*Kinosternon odoratum*					
	10 ♂ ♀	0				
	10 ♂	0				
	20 ♂ ♀	1				
	50 ♂ ♀	1-2				
Amphibians	*Rana pipiens* ♂		*Rana pipiens* ♀ *and* ♂ ♀			
	6	0	4 ♀	0		
	4	0	8 ♀	?		
	24	2	100 ♂ ♀	?		
	8	?	60 ♂ ♀	1?		
Fish ♂ ♀	*Ameiurus nebulosus*		*Eupomotis gibbosus*		*Various*	
	48	2	30	0-1	1 tile fish	?
	80	2	50	3	1 mackerel	?
	48	traces	30	?	2 salmon	0
	100	2?			15 perch	0
	100	4			4 codfish	0
	70	2?			2 ,,	0
	50	?			5 ,,	?
					4 ,,	0

The sign ♂ ♀ indicates that males and females have been used without regard to sex.

vertebrates, also with livers of all submammalian classes. That this reaction implies the presence of the lactogenic hormone may be doubted because of the absence of fat granules in the proliferations.

9294

Selenium Poisoning in Fishes.*

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In studies on the toxicity of selenium we have found marked pulmonary edema in dogs following the intravenous injection of sodium selenite equivalent to 1.83 mg. of selenium per kg., and in rats following intraperitoneal injections equivalent to 3.7 mg. of selenium per kg. As these findings on dogs and rats, and the various published observations on the volatile odoriferous compounds in the expired air from man[1] and other mammals[2] which have received small quantities of selenium, direct attention to the lungs in selenium poisoning, a series of studies on selenium poisoning in gill-breathing fishes has been made.

One hundred fifty goldfish (average length *circa* 80 mm.) were carried in individual glass jars each containing 4 liters of well aerated tap water to which known quantities of sodium selenite were added. Analyses at frequent intervals showed that the quantities of sodium selenite used made no appreciable changes in the dissolved oxygen, pH, or conductivity of the water. Each fish was changed to fresh seleniferous water every 48 hours and fed 3 standard pellets of shrimp meal immediately after being transferred.

In waters containing 2 parts per million of selenium the fish showed no symptoms or distress for the first 8 days, taking their food promptly. On the 8th day of exposure to 2 ppm. of selenium the fish began to refuse food, or if food were taken to regurgitate it quickly. This reaction was followed by marked anorexia for a period of several days. The first fish in this series died on the 18th day of exposure to 2 ppm. of selenium and deaths became progressively more frequent between the 25th and 37th days of the experiments, the longest survival being 46 days. The onset of anorexia always marked the beginning of the fatal phase of selenium poisoning. During the next few days the fish frequently showed incoordination and a definite disturbance of equilibrium and as the poisoning progressed became more lethargic and feeble. These changes in behavior were not due to lack of food alone, for these

* Published by permission of the Commissioner of Fisheries.

1 Dudley, H. C., *Am. J. Hygiene*, 1936, **23**, 181.

2 Franke, K. W., and Moxon, A. L., *J. Pharm. Exp. Ther.*, 1936, **58**, 454.

symptoms did not develop in a comparable unfed control series of fish not exposed to selenium. In other tests in which the surrounding water carried 5 ppm. of selenium goldfish died in from 4 to 10 days, the sequence of poisoning being the same as in 2 ppm. series. The goldfish experiments showed that selenium could be taken up in cumulatively lethal quantities by fish from the surrounding water.

As the actual selenium intake was not determined in the goldfish series, known quantities of sodium selenite (in a 0.5% solution or less) were injected intraperitoneally into 550 catfish, *Ictalurus punctatus,* averaging 160 mm. in length and 54 gm. in weight. Control fish given comparable injections of physiological saline developed no symptoms and remained healthy throughout the tests. All fish were bathed in salt solution immediately after each handling to prevent fungus infections, and held out-of-doors (these experiments were conducted at Ft. Worth, Texas) in concrete hatchery raceways through which unpolluted water flowed continuously.

Two types of reactions were obtained from single injections of sodium selenite, namely, acute and delayed. Sodium selenite in excess of *circa* 3 mg. of selenium per kg., *i. e.,* 0.15 mg. of selenium per fish was fatal in less than 48 hours, usually in less than 7 hours at water temperature around 10°C. The toxicity of the selenite increased markedly with temperature as 0.35 mg. of selenium per kg., *i. e.,* 0.018 mg. per fish killed in 24 hours or less at 27°C. Fish dying from acute selenium poisoning consistently showed contraction of the dermal chromatophores, so that fish receiving selenium injections were definitely lighter in color often for several hours before death than control fish receiving comparable quantities of physiological saline. This reaction of the chromatophores was readily produced locally on any part of the body by subcutaneous injections of selenite. Shortly before death the poisoned fish developed incoördination and often made spasmodic movements.

At any given water temperature the break between the dose of selenite producing these acute effects and that producing delayed effects was quite abrupt. Fish receiving less than the immediately toxic dose of selenite showed no symptoms for 4 to 10 days after the injection. However, about the 7th day after injection in most cases the eyes began to protrude and the abdomen became more or less pendulous. These conditions grew progressively worse for the next several days at which time the eyes of many fish were almost extruded from the head. Autopsies of fish dying in this "pop-eye" condition of extreme exophthalmus showed the body cavities to be more or less distended with ascites often containing blood, and the

periocular spaces to be filled with highly edematous tissue, that is, the eyes had been extruded by pressure from behind.

Fish in this "pop-eye" condition usually died about 20 to 25 days after receiving the injection of selenium. At water temperatures of 12-13°C. a single injection of 0.9 mg. of selenium per kg., or about 0.05 mg. per fish, was sufficient to produce fatal cases of this "pop-eye" disease, and smaller amounts were effective at higher temperatures as has been noted in connection with the quantities producing acute lethal poisoning.

At water temperatures of 10-13°C. daily injections of 0.04 mg. per kg. of selenium that is, 0.002 mg. per fish produced the exophthalmus after 5 injections, the fish having received a total of 0.01 mg. of selenium.

Blood studies made on 19 catfish, 6 normal and 13 in various stages of selenium "pop-eye" disease showed that although the red blood cell count in the selenium poisoned fish averaged less than one-half million below the normals (2,053,000 normal; 1,847,000 selenized fish), the total hemoglobin of the poisoned fish (Sahli Method) was about 3 gm. per 100 cc. below normal, (normal 9.8 gm., selenized fish 6.9 gm. per 100 cc.). As this hemoglobin level is far out of proportion to the slight lowering of the red blood cells found, it suggests that the selenium was interfering with normal hemoglobin formation. In the selenium-treated fishes many immature red blood cells were present in the blood, indicating a disturbance of the hematopoietic functions. The white blood cell count averaged about 4,000 per cu. mm. higher in the selenium-poisoned fish than in the normal, (14,200 normal, 18,300 selenized fish), and differential counts showed a lymphocytosis from normal to 82% of all white cells.

The blood of the selenium poisoned fishes was also more watery than that of normal fishes, the average specific gravities (Barbour method) being 1.0247 and 1.0338 respectively.

The microscopic pathology of catfish receiving selenium showed extensive areas of degeneration in the liver about the central veins. For the most part nuclei were preserved, but seemingly were pushed apart by the excessive accumulation of fluid, which in some areas had disrupted about one-half of the liver pulp cells. Hyaline degenerative changes were present in the walls of the hepatic arteries.

The spleen was extensively infiltrated with fluid which spread the elements apart, with dilatation of the smaller blood vessels and capillaries. The fibrous tissue was increased, replacing the lymphoid elements, and the capsule was distended by fluid in the subcapsular

region, which in many areas separated it from the underlying tissue.

The mesonephros had wide separations of the glomerular and tubular elements, by the excessive interstitial accumulation of fluid. The cellular elements were definitely pale in appearance as compared to the normal due to intrastitial accumulation of fluid, although the tubules were not disrupted. Many glomeruli showed accumulation of fluid between the glomerular tuft and Bowman's capsule.

The edema of the stomach was the most extensive of any of the organs studied. The submucosa showed the greatest change, with wide dilatation of capillaries, arterioles and venuoles. The accumulation of fluid increased the width of the submucosa approximately 4 times, making it about twice as thick as the mucous membrane. The mucous membrane was detached from the underlying submucosa in some areas, apparently being pushed out by the fluid below. The muscle layers of the wall showed considerable edema, with separation of the muscle fibers, especially in the longitudinal group. Similar changes but less severe in degree were found in the small intestine. Some sections showed excessive accumulation of fluid beneath the serosa, tending to separate it from the muscular layer.

The ovaries showed extensive intercellular accumulation of fluid, the degree of edema being very pronounced.

The spaces along the cartilaginous supports of the gill filaments were dilated with fluid and some of the gill filament processes were swollen and distorted. The striated muscle bands at the base of the filaments were edematous with separation of the fiber bundles.

The pathology of the various organs revealed an upset in permeability with excessive accumulation of fluid giving rise to edema in all of the structures studied. Apparently the edema was more extensive in the stomach than in any of the other organs, with the greatest involvement in the submucosal layer.

9295 P

Attenuation of Insulin by Interfacial Adsorption.*

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In a large series of experiments carried out on a group of 8 rabbits (average weight about 3 kilos) it was found that the physiological behavior and physical properties of crystalline insulin† are markedly altered by interfacial adsorption, so that a prolonged action of the insulin is acquired. The change is apparently that of denaturation such as proteins undergo at interfaces. Experiments have been largely of an exploratory nature to determine the most desirable mode of procedure but the results thus far obtained definitely indicate the effect which such adsorption has on insulin.

Aqueous solutions of crystalline insulin (U 40) having a pH of 2.5, were emulsified with equal volumes of Merck's Blue Label chloroform by means of an electrically driven shaking device. While other water insoluble volatile organic liquids can be used and have been used in these and other similar experiments, chloroform was chosen because of the purity of the material available, the ease with which it forms stable emulsions with protein solutions, and the ease with which it can again be removed by subsequent evaporation under reduced pressure.

The procedure used in emulsification can naturally be varied as to the length of time of shaking and of subsequent standing before recovery of the insulin solution. The optimum conditions need still to be determined. In the experiments which here illustrate the effects of emulsification, shaking was continued for 18 hours and the insulin solution recovered immediately by completely evaporating the chloroform at 45° under reduced pressure.

Following evaporation of the chloroform the insulin solution was strongly opalescent although its pH was practically unchanged. Centrifugation usually leaves an opalescent supernatant liquid over the insoluble residue which is thrown down.

The comparative physiological effect, expressed in milligrams percent of blood sugar in rabbits, of untreated insulin and that recovered

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† The generosity of the Eli Lilly Company which donated this material is gratefully acknowledged.

in both the supernatant liquid and the insoluble residue of insulin treated by interfacial adsorption, is shown in Table I, where the prolonged action of the treated insulin may be observed. The insulin in each case was injected subcutaneously.

TABLE I.

Hours	0	1	2	3	4	5	6	7	8	10	12	13	18	22	25	30
A	89	31	30	33	52	82	93	93								
B	82	63	56	59		65		74			78					
C	83	42	36	41	42		51		55			56	80	78	80	83
D	87	78	71	77	77	74	75	68		80	78					

A represents the standard dose of crystalline insulin required to bring the blood sugar of a rabbit to the convulsive level. B is a dose of supernatant fluid representing 3 times the original volume of untreated insulin normally used as in A. C represents the insoluble fraction of 30 units of crystalline insulin after denaturation. D represents 5 times a normal dose of insulin contained in a mixture of the supernatant liquid and insoluble residue of another sample of treated insulin.

Variations in the methods of procedure are being tried to note whether the apparent decrease in activity per unit of insulin can be prevented while retaining its prolonged activity after injection.

As will be shown elsewhere, this method has also been used to bring about the attenuation of bacterial toxin.

9296 P

A Colorimetric Assay for Male Sex Hormones in Urine.

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Zimmerman[1] described a color reaction for the R-CH_2-CO-R group of the sex hormones using meta dinitrobenzene as his reagent. Although the reaction is not specific the author after some preliminary trials has been able to adapt it so that it can be used as an index to the male sex hormone content of urine and as a guide for capon assays of urinary extracts where more complete characterization of the extract is desired.

Capon assays were performed using the alcoholic inunction technique described by Fussgänger[2] and elaborated by Dessau.[3] The

* General Education Board Fellow.

1 Zimmerman, W., *Z. f. Physiol. Chem.*, 1935, **233**, 257.

2 Fussgänger, R., *Medicine in its Chemical Aspects*, 1934, **2**, 185.

3 Dessau, F., *Acta Brev. Neer.*, 1935, **5**, 139.

growth of the comb was determined by measuring with a planimeter the area of a shadow photograph of the comb taken under standard conditions. The author's standard deviation, calculated from 5 measurements each on a group of 10 capons, according to the statistical methods of Fisher,[4] was ±3.9%. Growth of combs is expressed in percent increase in area using the size of the starting comb as the baseline. Since the relation between comb growth response and the color assay on urine extracts is the important feature of this study no effort is made to describe the value of the color unit in gammas of androsterone. It is generally agreed that the inunction assay for androsterone is about 50 times as sensitive as the intramuscular injection technique.

A 24-hour specimen of urine is brought to a pH below 1.0 with concentrated sulfuric acid and autoclaved for 15 minutes at 15 pounds pressure. After cooling, the urine is extracted with benzene in a continuous extractor until extraction is complete. The benzene is distilled off and the residue is dissolved in about 100 cc. of ethyl ether. This is transferred to a separatory funnel where the ether solution is extracted 5 times with 25 cc. portions of 10% aqueous solution of sodium hydroxide. The ether solution is then washed 3 times with 30 cc. portions of distilled water and transferred to a beaker, where it is stirred with 0.5 gm. of Norit (decolorizing charcoal) and filtered into a 125 cc. distilling flask. The charcoal is washed once with fresh ether. The ether solution at this point should be practically free from color. The ether is then distilled off and 20 cc. of 60% ethyl alcohol solution (60 cc. of 95% alcohol diluted to 95 cc.) is carefully measured into the flask to dissolve the hormone residue.

Five cubic centimeters of this alcohol solution, or less of it diluted to 5.0 cc. with 60% alcohol, is placed in one of the tubes of the colorimeter and 1.0 cc. of a 2% alcoholic solution of meta dinitro-benzene (2.0 gm. per 100 cc. of 95% alcohol) is added. One cubic centimeter of a 15% aqueous solution of KOH (15.0 gm. per 100 cc. of distilled water) is added, the tubes are shaken and set aside for an hour and a half in a dark corner of the room. A blank tube containing 5 cc. of 60% alcohol plus the reagents is prepared along with the unknowns. The color is measured in a simple colorimeter prepared for this purpose under the direction of the author by the Hellige Manufacturing Company. Color is expressed directly in color units read from the color disc of the colorimeter and the number of color units per 24-hour specimen is calculated.

[4] Fisher, R. A., 1930, Statistical Methods for Research Workers, 283 pages.

The alkali treatment of the ether solution removes the female sex hormones and the use of charcoal in small amounts does not remove male hormone activity to any significant extent. This point has been tested several times by capon assays.

Table I summarizes the data of a series of assays comparing the colorimetric assay with the comb growth assay. The latter is expressed in percent increase in comb area after 5 daily applications of 0.3 cc. of the alcoholic hormone solution to the combs of at least 2 capons. Each comparison in the table is a specimen of urine from a different individual.

TABLE I.

Comparison of the Colorimetric and the Inunction Assays of Urine Extracts for Male Sex Hormone.

The comb growth response is expressed as the average percent increase in area after 5 daily applications of 0.3 cc. of the alcoholic hormone solution to the combs of at least 2 capons.

Color Units per 24 Hour Specimen	Comb Growth Response
Units	%
Doubtful	4.5
3.2	5.0
11.2	17.4
24.0	18.0
27.0	28.0
40.0	42.0
56.0	36.0
64.0	25.0
80.0	33.0
96.0	38.0
104.0	63.0
112.0	58.0
112.0	68.0
112.0	84.0
120.0	45.0
120.0	68.0
144.0	120.0
152.0	55.0
168.0	77.0
200.0	87.0

These data show a satisfactory correlation between the two methods of assay. In examining the data it should be remembered that the color developed is not specific for male sex hormones. The author believes, however, that the colorimetric assay can be used in clinical studies with as much confidence as is now placed in the comb growth assay using intramuscular injections. The colorimetric assay is decidedly more sensitive to changes in the hormone content of urine and requires a less complicated set-up.

Summary. A colorimetric method is described for male sex hormone assays on urine. The color assays are compared with comb growth assays on capons.

9297

An Empirical Regression Equation Relating Total Serum Calcium to Serum Albumin and Globulins.

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A number of empirical regression formulae have been proposed to express the relation between the level of total calcium in the serum and that of the serum proteins.[1] These equations relate total serum calcium to *total* serum protein and take the general form:

I. Total Ca $= m$. total protein $+ b$ (where m and b are constants).

In the present study we have evaluated statistically the applicability of this general equation to our own data, consisting of 164 observations on 123 cases presenting a wide variety of changes in serum proteins.* The mean ratio: total serum calcium *calculated*/total serum calcium *observed* was calculated at different total protein levels, using values for m and b suggested by various investigators[1] and such other values as seemed appropriate.

A similar analysis was made of the general regression equations:

II. Total Ca $= m_1$. albumin $+ m_2$. globulin $+ b$ (where m_1, m_2 and b are constants).

and III. Total Ca $= m$. albumin $+ b$ (the special case where $m_2 = 0$).

As no constants for equations II and III could be found in the literature, values giving the best fit with our data were calculated.

Results. Equation I gives good agreement between calculated

[1] McLean, F. C., and Hastings, A. B., *J. Biol. Chem.*, 1935, **108**, 285.

* Our data include 27 observations on 21 cases of the nephrotic syndrome, with low albumin and normal or somewhat decreased globulin levels; 20 observations on 15 normal subjects; 50 observations on 39 cases of lymphogranuloma inguinale, 38 sera containing more than 8.0% total protein; 25 observations on 20 miscellaneous cases with hyperproteinemia not due to lymphogranuloma inguinale, multiple myeloma or cirrhosis; and 42 observations on 28 cases of hepatic cirrhosis, with low or normal albumin and normal or high globulin levels. Cases of extreme dehydration were not available for study.

All cases with a primary disturbance in calcium metabolism or with hyperphosphatemia were excluded. Multiple myeloma was not included because it is characterized by bone destruction (a primary disturbance in calcium metabolism). Cases of hypoproteinemia due to malnutrition or cachexia were excluded because the assumption of a constant Ca^{++} concentration—an assumption necessary because *total*, not protein-bound calcium values are used—is unwarranted in a condition where Ca^{++} may be so reduced that tetany results.

and observed total calcium values where the total protein is low or normal, but increasingly divergent results in hyperproteinemia if the means of the empirical values for m and b in the literature[1] are used (Table I). By decreasing m, b remaining constant (or by decreasing b, m remaining constant), calculated calcium values approach observed calcium values more closely in hyperproteinemia, but become too low in normal and nephrotic sera. It was not possible, by varying m or b or both in equation I, to obtain calculated calcium values in satisfactory agreement with observed calcium values for sera with normal globulin content and, at the same time, in hyperglobulinemia (Table I).

TABLE I.

Results of application of equations I, II, III and IV to our data, grouped according to total serum protein content. Representative mean ratios: $\frac{\text{total serum Ca calculated}}{\text{total serum Ca observed}}$ obtained by trial of various constants in these equations are shown. The ratios should be 1.00.

Formula Used	Constants Used	Mean Ratio $\frac{\text{Total Ca calculated}}{\text{Total Ca observed}}$ Total serum protein (gm. per 100 gm. H_2O)				
		3.1-6.0 (28 obs.)	6.1-8.0 (20 obs. normals)	6.1-8.0 (30 obs. patients)	8.1-10.0 (68 obs.)	10.1+ (18 obs.)
I.	Ca =.75 total protein +5.6	1.03	1.02	1.13	1.16	1.29
	Ca =.56 " " +5.6	0.94	0.89	0.99	1.00	1.09
	Ca =.75 " " +4.0	0.87	0.87	0.97	1.01	1.14
	Ca =.60 " " +6.0	1.01	0.95	1.06	1.07	1.18
II.	Ca =.83 albumin + .4 globulin +5.9	0.99	1.00	1.00	1.01	1.10
	Ca =.83 albumin +.25 globulin +6.0	0.96	0.97	0.99	0.99	1.01
III.	Ca =.80 albumin +7.1	1.01	1.00	0.95	0.96	0.93
	Ca =.80 " +7.2	1.02	1.00	0.96	0.97	0.94
	Ca =1.1 " +5.8	0.94	1.01	0.94	0.95	0.91
IV.	Ca =.80 albumin +.2 "globulin I" +7.0	1.00	0.99	0.99	0.99	1.00

In equation I, a common factor (m) is used for calcium bound to albumin + globulin (*i. e.*, total protein). The ratio: albumin/globulin does not remain constant, however, as the total serum protein content rises above or falls below normal levels; *hyper*proteinemia being due to an increase in the *globulin* fraction, *hypo*proteinemia chiefly to a fall in the *albumin* fraction.[2] The term 'm . total protein', therefore, could be employed both in hyper- and hypoproteinemia only if the albumin fraction and the total globulin fraction

[2] Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 511.

bound approximately the same amount of calcium per gram. It appears from our analysis of equation I (Table I) that a common factor (m) cannot be so employed. At least in hyperglobulinemia, the amount of calcium bound per gram albumin appears to differ appreciably from that bound per gram total globulin under the conditions existing in these sera.

Equation II, unlike equation I, allows for differences in calcium bound per gram albumin and calcium bound per gram globulin and gives much better fit with our data. Nevertheless, it was not possible to find values for m_1, m_2 and b such that satisfactory agreement between calculated and observed calcium values was obtained both when serum globulin was normal and when it was definitely increased (Table I). These discrepancies result because the globulin fraction is itself heterogeneous; hyperglobulinemia being almost invariably due chiefly or wholly to increased euglobulin, as determined by Howe's method. A common factor (m_2) therefore could be used for the several serum globulins over the range of variation in total globulin content only if the several serum globulins bound approximately the same amount of calcium. The discrepancies encountered with equation II suggest that the several serum globulins bind different amounts of calcium under the conditions existing in these sera.[2, 3]

Equation III, which appears to imply that essentially all protein-bound calcium is bound to albumin, does not fit our data well in cases with marked hyperglobulinemia. The chief difficulty with this equation, however, is that b, which represents the amount of calcium not bound to protein and should be approximately 5.8 mg. per 100 gm. serum H_2O (the mean of determined values for diffusible calcium) must approach 7.0 if calculated values for total calcium are to approximate observed total calcium values. We interpret this discrepancy to mean that the constant 7.0 really is the sum of 2 constants; one of which (5.8) represents calcium not bound to protein, the other (approximately 1.2) represents calcium bound to protein but not to albumin. The latter calcium fraction is bound, presumably, to a globulin fraction ("globulin II"), and since it is a constant, remains of the same order of magnitude irrespective of increases in total globulin content. "Globulin II" roughly approximates, statistically, the pseudoglobulin II fraction as determined by Howe's method. The remaining globulin fraction, "globulin I", the globulin increment responsible for hyperglobulinemia (usually chiefly

[3] Gutman, A. B., Gutman, E. B., Jillson, R., and Williams, R. D., *J. Clin. Invest.*, 1936, **15**, 475.

euglobulin as determined by Howe's method) appears to bind so little calcium as to be insignificant except at high total globulin levels.

Conclusions. The above considerations suggest that the total serum calcium is composed of at least 4 fractions: 1. Calcium bound to and varying with albumin; 2. calcium bound to a globulin fraction, relatively constant in amount irrespective of the total globulin level; 3. calcium bound to another globulin fraction, varying with that globulin fraction, and though small, becoming significant at high total globulin levels; 4. calcium not bound to protein, relatively constant because cases with gross variations are excluded by definition. These fractions are represented in the following general regression equation:

IV. Total Ca $= m_1$. albumin $+ m_2$. "globulin II" $+ m_3$. "globulin I" $+ b$.

Where $b = 5.8 \pm 0.2$ mg. per 100 gm. serum H_2O, analysis of our data suggests that m_1 is of the order 0.7-0.9 mg. Ca per gram albumin; the product m_2 . "globulin II" is a constant of the order 1.0-1.5 mg. Ca per 100 gm. serum H_2O; m_3 is of the order 0.1-0.2 mg. Ca per gram "globulin I" where "globulin I" is defined arbitrarily as all globulin in excess of 3.0 gm. total globulin.

While the method does not warrant further precision of constants, it is of interest that a number of formulae based on equation IV may be devised which tend to obviate the systematic divergencies of equations I, II and III. Table I and Fig 1 illustrate the results where:†

V. Total Ca $= .80$ albumin $+ 7.0 + 0.2$. (total globulin—3.0).

Calcium is expressed in mg., albumin and globulin in gm. per 100 gm. serum H_2O. The constant 7.0 is the sum of b and m_2 . "globulin II". The last term is used only if the total globulin content exceeds 3.0 gm.

The fact that equation IV applies more generally to our data is regarded as support for the inferences drawn concerning calcium bound to the several protein fractions binding calcium and the approximate amounts so bound. It should be emphasized that no special significance is attached to the particular values used by us in equation V except as they are indicative of approximate orders of magnitude. We do not believe that a discrepancy between observed calcium values and those calculated by this or other equations neces-

† These constants are derived from sera of adults. The formula is not applicable where there is a primary disturbance in calcium metabolism, hyperphosphatemia or severe malnutritional hypoproteinemia, in which conditions gross variations in Ca^{++} occur. Appreciable variations in Ca^{++} concentration may occur in any wasting disease and constitute a source of error.

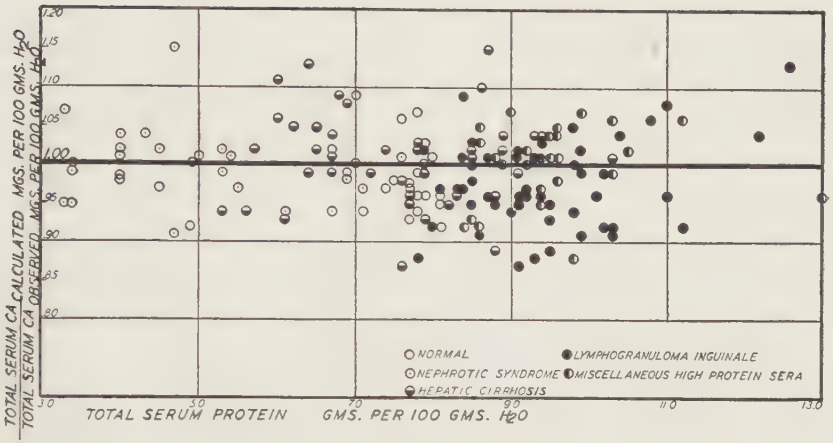

FIG. 1.

Correlation between observed Ca and Ca calculated by equation V for our data on 164 sera. The standard error of estimate is 0.575 mg. Ca. 65% of the mean ratios are within ±5% of 1.00; in 20 instances the mean ratio exceeded 1.05, of which 5 exceeded 1.10; in 32 instances the mean ratios were less than 0.95, of which 7 were less than 0.90. More symmetrical distribution but less satisfactory agreement with data in the literature is obtained if 7.1 is used as a constant instead of 7.0.

sarily implies the presence of a primary disturbance in calcium metabolism. As a prediction formula, equation V is of academic interest only, since total serum calcium may be determined directly with precision.

Methods. Serum calcium was determined by the Clark and Collip modification of the Kramer and Tisdall method. Serum protein was determined by difference, total N by the Kjeldahl technique, non-protein N by Folin's method with Nesslerization. Albumin and the globulin fractions were estimated by Howe's method, N being determined by the micro-Kjeldahl technique and titration. Determinations were made in duplicate, except some calcium determinations when insufficient serum was available. Corrections for specific protein volume were made[1]: 99.0—0.75 total protein.

9298

Comparative Effectiveness of Various Chemical Sprays in Protecting Monkeys against Nasally Instilled Poliomyelitis Virus.

Peter K. Olitsky and Albert B. Sabin.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

We reported[1] the protective action of sodium alum and tannic acid instillations against nasally instilled poliomyelitis virus in *Rhesus* monkeys. It was pointed out that the action of these chemicals was dependent on their concentration and was exerted on the nasal mucosa of the host rather than on the virus, although it appeared from one experiment that the potency of the test dose of virus influenced the results. Upon resuming this work at a later date we were unable to repeat our original results with sodium alum. Investigation of this failure disclosed the participation of the following factors: (1) the sodium alum which we used in the original experiments was assumed to have 24 molecules of water of hydration (as indicated by the manufacturers' label) while actually it possessed only 2 molecules and contained some insoluble Al_2O_3; (2) the sodium alum used in subsequent experiments really had approximately 24 molecules of water of hydration, had no Al_2O_3 and gave a clear solution, so that the actual amount of $Na_2SO_4 \cdot Al_2(SO_4)_3$ in a 4% solution of this preparation was in the ineffective range; (3) that the virus used in the subsequent experiments had become for some unknown reason considerably more potent; while originally only about 80% of control monkeys developed poliomyelitis, all untreated animals now succumbed even when the dose of virus was reduced.

In an attempt to elucidate further the mechanism of the protective action of these chemicals as well as to find the most effective substance, experiments were performed with a number of astringents which are used in human beings for one purpose or another. Rather than reduce the test dose of virus to a point where only a majority of untreated monkeys would develop poliomyelitis and have a larger number of agents exhibit protective properties, the more highly infective amount of virus was used (regularly paralyzing all untreated monkeys) to discover whether under these conditions any of the substances would still be effective. It was furthermore realized

[1] Sabin, A. B., Olitsky, P. K., and Cox, H. R., *J. Bact.*, 1936, **31**, 35; *J. Exp. Med.*, 1936, **63**, 877.

that a reliable comparison of the effectiveness of various chemicals can be made only when all the substances to be compared are used in a single experiment under identical conditions of treatment and infection—for even with 100% infectivity the potency of various virus preparations can vary in different experiments.

The astringent solutions were sprayed into the nostrils by hand with a DeVilbiss atomizer, delivering approximately 1.5 cc. of the solution in each side of the nose by means of 10 to 15 bulb compressions. The monkeys were thus treated once a day for 7 days, and 2 days after the last spraying poliomyelitis virus was instilled into the nose. The monkeys were then observed for a month, and those which survived and failed to develop paralysis were, without any additional chemical treatment, again instilled with virus, the processes being repeated until practically all monkeys became paralyzed, thus indicating the duration of chemical protection. The virus consisted of pooled glycerolated bits of spinal cords from 4 to 6 paralyzed monkeys, ground up to a 5% suspension in physiological saline solution and centrifuged only enough to eliminate the alundum and large tissue particles. One cc. of this suspension was dropped into each nostril in the morning and then again in the afternoon of the same day.

The following substances were studied: (1) 4% tannic acid; (2) 4% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$ including suspended Al_2O_3; (3) 7% $Na_2SO_4 . Al_2(SO_4)_3 . 24H_2O$; (4) 7% $K_2SO_4 . Al_2(SO_4)_3 . 24 H_2O$; (5) 0.5% picric acid —0.5% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$; (6) 1% $ZnSO_4 . 7H_2O$ (granular and crystalline); (7) 5%

TABLE I.

Chemical used	Days after last chemical treatment poliomyelitis virus instilled in nose							
	2		30		64		93	
	Polio	No Polio	Polio	No Polio	Polio	No Polio	Polio	No Polio
Untreated controls	8	0	4	0	3	0	–	–
4% tannic acid	0	4	2	2	2	0	–	–
1% $ZnSO_4 . 7H_2O$ (granular)	0	4*	0	1	1	0	–	–
5% $FeSO_4 . 7H_2O$	1	3†	0	2	1	1	0	1‡
0.5% picric acid— 0.5% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$	1	3	3	0	–	–	–	–
4% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$ (including suspended Al_2O_3)	2	2	2	0	–	–	–	–
7% $Na_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	1	2	2	0	–	–	–	–
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	1	3	0	3	1	2	0	2‡

* Three of these 4 monkeys and one of the 3 marked † died of tuberculosis at the end of one month and showed no microscopical evidence of poliomyelitis.

‡ These 3 monkeys are being studied to determine whether they developed specific immunity as a result of the repeated virus instillations which they resisted.

$FeSO_4 . 7H_2O$; (8) Iron Dialyzed (Colloidal) Merck—undiluted, and (9) diluted 1:3. Including untreated controls, 104 monkeys were used. The results are presented in 4 tables, each table containing the data of a series of simultaneous tests.

In the first series of tests (Table I) tannic acid, $ZnSO_4 . 7H_2O$, $FeSO_4 . 7H_2O$, and $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$ emerged as highly efficient agents on the basis of the 2- and 30-day tests; the sodium alum solutions and the picric acid mixture had some effect when the virus was given 2 days after the last chemical treatment but at 30 days all the monkeys succumbed. Without any change in procedure or technique except perhaps for an unknown increase in the potency of the virus, subsequent tests (Tables II, III, IV) further eliminated tannic acid, potassium alum, ferrous sulphate, and colloidal

TABLE II.

Chemical used	Days after last chemical treatment poliomyelitis virus instilled in nose			
	2		49	
	Polio	No polio	Polio	No polio
Untreated controls	6	0	4	0
4% tannic acid	6	0	–	–
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	5	0	–	–
5% $FeSO_4 . 7H_2O$	5	1	1	0
1% $ZnSO_4 . 7H_2O$ (granular)	1	5	3	2

TABLE III.

Chemical used	Days after last treatment poliomyelitis virus instilled in nose			
	2		30	
	Polio	No polio	Polio	No polio
Untreated controls	3	0	4	0
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	2	1	–	–
1% $ZnSO_4 . 7H_2O$ (granular)	0	3	0	3

TABLE IV.

Chemical used	Virus 2 days after last chemical treatment	
	Polio	No polio
Untreated controls	4	0
1% $ZnSO_4 . 7H_2O$ (crystalline)	0	4
4% tannic acid (Baker and Adamson)	2	1
4% tannic acid (Mallinckrodt)	2	1
Dialyzed (colloidal) iron (Merck) undiluted	3	1
Colloidal iron—1 part + Distilled water—2 parts	4	1

iron, leaving zinc sulphate as the most effective protective agent of all the substances investigated. This high efficiency of zinc sulphate is in agreement with recent observations of Schultz and Gebhardt.[2] With this very potent test dose of virus zinc sulphate protected 16 of 17 monkeys tested at 2 days, all of 4 at 30 days and 2 of 5 at 49 days after the last chemical treatment.

9299

P-Aminobenzenesulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats.

PAUL GROSS AND FRANK B. COOPER. (Introduced by R. R. Mellon.)
From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh.

Although Hörlein[1] claimed that Prontosil was effective against Type III pneumococcal infections, experimental proof for this assertion was lacking until Rosenthal[2] and Cooper, Gross, and Mellon[3] independently investigated this problem.

They demonstrated that *p*-aminobenzenesulfonamide gave mice a certain degree of protection against lethal doses of the particular strains of Type III pneumococcus employed. Buttle, Parish, McLeod, and Stephenson,[4] however, were unable to demonstrate any significant protection in mice infected with Types I and II pneumococci, whereas Rosenthal[2] obtained protection against all 3 fixed types.

The lack of parallelism between pneumococcal septicemia in mice and pneumonia in man led to the choice of the experimental pneumococcal pneumonia in the rat[5, 6] as a closer approximation to human pneumonia. The encouraging results obtained by treating such experimental Type III pneumonia with *p*-aminobenzenesulfonamide[7, 8]

2 Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 524.

1 Hörlein, H., *Proc. Royal Soc. Med.*, 1936, **29**, 321.

2 Rosenthal, S. M., *Public Health Reports*, 1937, **52**, 48.

3 Cooper, F. B., Gross, P., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 148.

4 Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

5 Nungester, W. J., and Jourdonais, L. F., *J. Bact.*, 1935, **29**, 34.

6 Gunn, F. D., and Nungester, W. J., *Arch. Path.*, 1936, **21**, 813.

7 Gross, P., and Cooper, F. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 225.

8 Cooper, F. B., and Gross, P., to be published.

have been duplicated in the treatment of human Type III pneumococcal pneumonia as reported by Heintzelman, Hadley and Mellon.[9]

The relative therapeutic efficacy of *p*-aminobenzenesulfonamide and of potent specific antipneumococcal serum was investigated in rats infected intrabronchially with the Type I (Neufeld) strain. The inoculum, 0.1 cc. of which was injected, consisted of an 18-hour broth culture diluted 1000 times with broth, and sufficient mucin (Armour) added to give a viscous solution.

Fifty-six rats, infected in this manner, were divided into 4 groups of 14 each: Group A—Untreated controls. Group B—Given 250 units of Type I antipneumococcal serum intraäbdominally 6 hours after infection, followed by 2 similar daily doses. Group C—Given

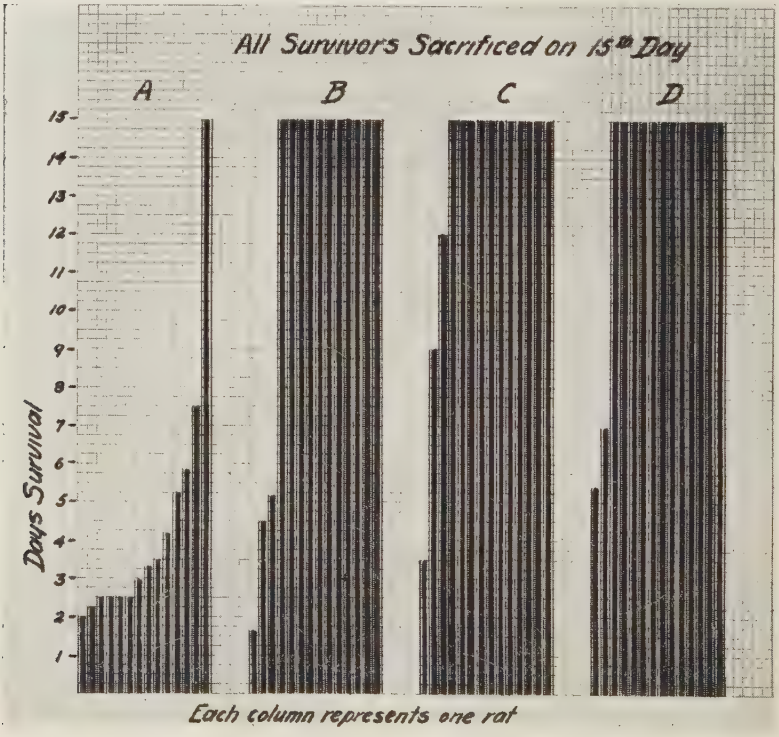

FIG. 1.

Graph of survival-time of rats infected intrabronchially with Type I pneumococcus.

A. Untreated controls.
B. Treated with Type I antipneumococcal serum.
C. Treated with *p*-aminobenzenesulfonamide by mouth.
D. Treated with both serum and *p*-aminobenzenesulfonamide.

[9] Heintzelman, J. H. L., Hadley, P., and Mellon, R. R., *Am. J. Med. Sci.*, in press.

125 mg. of *p*-aminobenzenesulfonamide* by mouth 6 hours after infection, followed by 10 daily similar doses. Group D—Treated 6 hours after infection by a combination of the treatments used in Groups B and C.

Animals surviving to the 15th day were killed with ether. All animals of each group were necropsied and smears from the blood (femoral vein), peritoneum, and pleura, were stained by Gram's method. Sections were made from all lobes of the lungs of each rat.

The effect of the various types of treatment on the survival-time and rate is shown graphically in Fig. 1. The gross and microscopic anatomic changes in the lungs, as well as the bacteriologic findings, are tabulated in Table I. A summary of the findings is given in Table II.

Contrary to the results reported by Gunn and Nungester[6] and by us,[7, 8] a lobar type of pneumonia developed in only a few rats. Curiously enough, the most extensive and fully developed lobar type was seen in the non-survivors of the treated groups.

Inflammatory changes were found in the lungs of every animal. Grossly, the changes were often minimal and not recognized. Bilateral empyema was frequently the outstanding postmortem finding. The most constant change was the interstitial pneumonia, more severe in the survivors than in the rats which died early. These interstitial changes represented, no doubt, the residues of subsiding pneumonias.

The reduction in the mortality-rate from 93% in the control group to 21% in the groups treated with serum or the drug, and to 14% in the group treated with both, clearly indicates that *p*-aminobenzenesulfonamide may have a definite place in the treatment of human Type I pneumococcal pneumonia in conjunction with specific antiserum; and particularly where antiserum is not available for economic or other reasons. Concomitant with the drop in mortality-rate there was a reduction in bacteremia, peritonitis (determined by the smears), and empyema (Table II).

The deaths in the drug-treated Group C occurred later than those of the serum-treated Group B, although they were numerically equal. Also, in the drug-treated group, 64% of the rats had no broncho- or lobar pneumonia, whereas only 50% of the rats in the serum-treated group were so spared. A rough approximation of the degree of microscopic involvement indicates less impairment in the group treated with *p*-aminobenzenesulfonamide than in the group treated with serum.

* Kindly supplied by Merck & Co., Inc., Rahway, N. J.

TABLE I.

Tabulation of necropsy findings. All animals which survived to the 15th day were sacrificed.

Treatment	No. Rat	Days Survival	Gross Pneumonia		Microscopic Pneumonia			Empyema	Peritonitis	Pneumococci in Smears of Blood Pleura Peritoneum		
			Right Lung	Left Lung	Lobar	Broncho	Interstitial					
None	1	2	—	—	—	+	++	—	—	+	+	—
	2	2	—	+	—	+	++	—	+	+	+	+
	3	2½	—	+	++	—	+	—	+	+	+	+
	4	2½	++	++	—	++++	—	+	+	+	+	+
	5	2½	—	+	+	+++	+++	—	+	+	+	+
	6	2½	++	—	++	+	++	—	+	+	+	+
	7	3	++	—	—	++	+	+	+	+	+	+
	8	3	—	—	—	+	+	+	+	—	+	+
	9	3½	—	+	—	+++	++	+	+	+	+	+
	10	4	—	+	—	+++	+	+	+	+	+	+
	11	5	—	—	++	++	++	+	+	—	+	+
	12	6	—	+	—	+	++++	+	+	—	+	+
	13	7½	—	+	—	++	—	—	+	—	+	+
	14	15	—	—	—	—	+	—	—	—	+	—
250 units serum, 3 successive days	1	1½	++++	+	++++	—	+	—	—	+	+	—
	2	4½	+	+	++	++	+	+	—	—	+	—
	3	5	—	++++	++++	—	++++	+	—	—	+	—
	4	15	—	—	—	—	++	—	—	—	—	—
	5	15	—	—	—	+	+++	—	—	—	—	—
	6	15	—	—	—	—	+++	—	—	—	—	—
	7	15	—	—	—	—	++	—	—	—	—	—
	8	15	++	+	—	++	+++	—	—	—	—	—
	9	15	++	+	—	+	+++	—	—	—	—	—
	10	15	—	—	—	—	+	—	—	—	—	—
	11	15	—	—	—	—	++	—	—	—	—	—
	12	15	—	+++	—	+++	+++	—	—	—	—	—
	13	15	—	—	—	—	+	—	—	—	—	—
	14	15	—	—	—	—	++	—	—	—	—	—

125 mg. *P*-amino-benzenesulfona-mide for 11 con-secutive days	1	3½	++	+	—	+	++	+	—	—	+	—
	2	9	—	+++	++++	++	+++	+	+	—	+	+
	3	12	—	++++	++++	.	—	+	—	+	+	—
	4	15	—	—	—	—	+	—	—	—	—	—
	5	15	—	—	—	—	++	—	—	—	—	—
	6	15	—	—	—	—	++	—	—	—	—	—
	7	15	—	—	—	—	+	—	—	—	—	—
	8	15	—	—	—	—	++	—	—	—	—	—
	9	15	—	—	—	—	+	—	—	—	—	—
	10	15	+	+	.	+++	++	—	—	—	—	—
	11	15	—	—	—	—	+++	—	—	—	—	—
	12	15	+	—	—	—	++	—	—	—	—	—
	13	15	+	—	.	—	++	—	—	—	—	—
	14	15	++	+	—	+	+++	—	—	—	—	—
Treated with both serum and drug as above	1	5½	—	—	—	++	+++	—	—	—	—	—
	2	7	++++	+	++++	—	—	—	—	—	+	—
	3	15	—	—	—	—	+	—	—	—	—	—
	4	15	—	—	—	—	++	—	—	—	—	—
	5	15	—	—	—	—	+	—	—	—	—	—
	6	15	—	—	—	—	++	—	—	—	—	—
	7	15	—	+	—	—	+++	—	—	—	—	—
	8	15	—	—	—	—	++	—	—	—	—	—
	9	15	—	—	—	+	+++	—	—	—	—	—
	10	15	—	—	—	—	++	—	—	—	—	—
	11	15	—	—	—	—	++	—	—	—	—	—
	12	15	—	—	—	—	++	—	—	—	—	—
	13	15	—	—	—	—	+	—	—	—	—	—
	14	15	—	—	—	—	+	—	—	—	—	—

TABLE II.
Summary of Results Listed in Table I.

	Group A Control	Group B Serum Treated	Group C *P*-amino-benzenesul-fonamide Treated	Group D Combination Drug and Serum Treated
	%	%	%	%
Mortality rate	93	21	21	14
Bacteremia	64	7	7	0
Peritonitis	86	0	7	0
Empyema	50	14	21	0
Absence of lobar or broncho-pneumonia microscopically	7	50	64	79
Degree* of microscopic pulmonary involvement	3.9	3.6	2.9	2.3

* These values represent the group-average of the number of plus signs found in the columns "Microscopic Pneumonia" of Table I.

It appears, therefore, that in the relative dosages employed in this experiment, the efficacy of the drug is as great as, if not greater than, that of the specific serum. The data also show that the best therapeutic results were obtained by a combination of serum and drug, indicating that the two methods of treatment are synergistic. On the basis of the excellent therapeutic results here obtained, it is suggested that this drug be tried in human Type I pneumonia in conjunction with the specific antiserum and particularly in cases where the antiserum is not available. This suggestion is supported by the parallelism which has been demonstrated with *p*-aminobenzenesulfonamide therapeusis of both rat and human Type III pneumococcal pneumonia and also by the fact that at times hemolytic streptococci are known to complicate pneumococcal pneumonias.

9300

Effect of Hypophysectomy on Blood Lactic Acid of *Rhesus* Monkeys.

ALEITA H. SCOTT. (Introduced by H. B. Williams.)

From the Department of Physiology, Columbia University.

Work on blood sugars in normal and hypophysectomized monkeys has been recently reported (Smith, *et al.*[1]). Blood lactic acid was simultaneously determined in many of these samples.

1 Smith, P. E., Dotti, L., Tyndale, H. H., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 247.

The material here reported was obtained from 15 normal, 11 completely hypophysectomized and 4 partially hypophysectomized monkeys. The blood sugar values for these individuals are also included for comparison. The blood lactic acid was determined by a modification of the method of Friedman, Cotonio and Shaffer (Scott[2]). Blood was drawn from the heart into a mixture of 50% NaF and Na-oxalate. All of the animals were starved from 16 to 18 hours.

TABLE I.
Blood Sugar and Blood Lactic Acid on Heart Blood Taken 16-18 Hours After Feeding, Both Before and After Complete Hypophysectomy.

Monkey No.	Before Operation No. of samples	Sugar mg. %	Lactic acid mg. %	After Operation No. of samples	Sugar mg. %	Lactic acid mg. %
251	1	86	151	2	50	55
253	3	118	167	5	57	27
258*	3	107	109	4	56	49
259	3	107	75	3	56	32
293	2	108	87	1	76	57

*Male castrate.

The high values for lactic acid in the monkey may be due to the muscular activity exerted by the animal before capture and to the muscular tension developed while the animal is being held during the drawing of the blood. The animals were chained so that there was a minimum of effort during their capture. However, the monkey in captivity is still a wild animal and opposes restraint.

Hypophysectomy is followed in the monkey by a fall in the blood sugar (Smith, *et al.*[1]). The blood lactic acid falls markedly also.

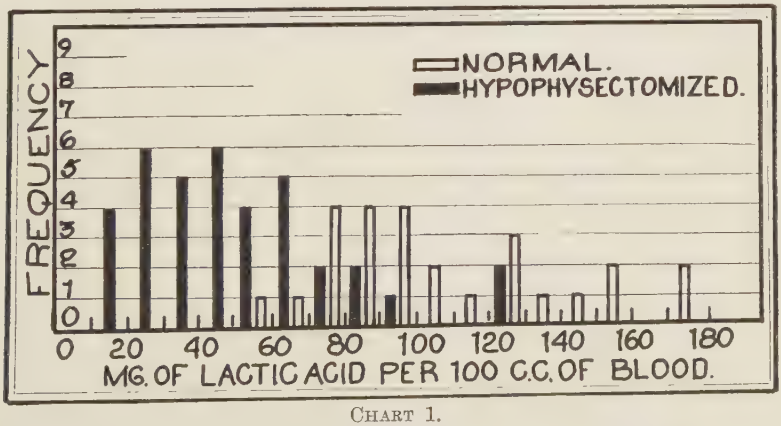

CHART 1.
Distribution of blood lactic acid in monkeys, starved 16-18 hours.

2 Scott, A. H., *J. Biol. Chem. Proc. Am. Soc. Biol. Chem.*, 1936, **8**, lxxxvii.

It is to be noted that this lower value of the blood lactic acid is still 5 times that for venous blood in man under basal conditions. There is, however a fairly good separation of the values in the normal and operated animals when arranged in a scatter chart (Chart 1). Those animals which were later found to have been incompletely hypophysectomized showed intermediate values for the lactic acid in like manner to that found to be true for the blood sugar (Smith, *et al.*)

TABLE II.
Summary of All Blood Lactic Acid Determinations.*
(Heart Blood, Animals Starved 16-18 hours).

	No. of samples	mg./100 cc.	ε	ε_M
	Normal Monkeys.			
Blood Lactic Acid	26	104	34.4	6.5
" Sugar	26	108	19.5	3.8
	Incomplete Hypophysectomy.			
" Lactic Acid	25	75	27.7	5.5
" Sugar	25	88	14.7	2.9
	Animal No. 261, 1 mm. tissue remaining after operation.			
" Lactic Acid	3	161	43.1	24.9
" Sugar	3	86	2.2	1.3
	Complete Hypophysectomy.			
" Lactic Acid	37	50	27.5	4.5
" Sugar	37	59	17.1	2.7

*The formula $\varepsilon = \sqrt{\frac{\Sigma d^2}{N-1}}$ was used, giving the mean deviation rather than the standard deviation (σ) because of the small number of cases studied. (Scott.[3])

Animal No. 261 was placed separately in the table. A small fragment of tissue one mm. in diameter was present after the operation. The blood sugar was about the same as for the incompletely hypophysectomized animals but the lactic acid was higher than the average of the normal monkeys.

[3] Scott, E. L., *J. Biol. Chem.*, 1927, **73**, 81.

9301

Is the Q-Factor of Body Segments Independent of Size and Shape?*

Avrom Barnett and Samuel Bagno. (Introduced by G. B. Wallace.)

From the Respiration Laboratory, New York Post-Graduate Medical School and Hospital.

One of the striking features of the methods proposed for the clinical measure of Q-factor[1, 2] is the apparent lack of relation between the values obtained and the physical conformation of the subject under test. No corrections for size, shape, height or weight are made. The tissues under investigation (arm-to-arm segment) are considered as a single piece of dielectric having a characteristic Q-factor and it is assumed that differences in size and shape have a negligible effect on the magnitude of the results obtained. It is proposed, in the present paper, to show that this assumption is unwarranted and that results based thereon are seriously open to question.

It may be noted at the outset that the Q-factor of a given material is, in general, independent of its size and shape only if it be electrically homogeneous. In considering the special case of the arm-to-arm segment, it is evident from the anatomy alone that we are dealing with a heterogeneous impedance composed of muscle, tendon, aponeurosis, fat, nerve, vascular tissue, etc., of which each component may be supposed to have special electrical properties. The work of Eyster and his co-workers[3] and of Katz and Korey[4] indicates that this is indeed the case. When low frequency alternating current transverses the thorax or the trunk, it distributes itself unevenly throughout these segments in proportion to the conductivities (admittances) of the component tissues, the least amount of current flowing in the membranous portions thereof—viscera and peritoneal cavity—and the greatest in the muscular masses. However, the data obtained by these authors were derived from measurements on animals and are limited to the abdominal and thoracic portions of the trunk. The frequencies involved are also of a different order

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Brazier, M. A., West, B., *J. Surg., Obst. and Gyn.*, 1935, **43**, 429.

2 Horton, J. W., and Van Ravenswaay, A. C., *J. Frank. Inst.*, 1935, **220**, 557.

3 Eyster, J. A. E., Maresh, F., and Krasno, M. R., *Am. J. Physiol.*, 1933, **106**, 547.

4 Katz, L. N., and Korey, H., *Am. J. Physiol.*, 1935, **111**, 83.

than those employed in Q-factor determinations. It is proposed to show that their findings apply equally well to the arm-to-arm segment and to man at frequencies in the vicinity of 10,000 cycles.

In approaching this problem, it may be noted, that it is not sufficient to show that the arm-to-arm segment is heterogeneous as to impedance alone. Obviously tissues of differing impedance may vary little as to Q-factor, *i. e.*, the ratio between their reactive and resistive components may still remain substantially the same. The heterogeneity of the arm-to-arm segment will, therefore, be considered not only as to impedance but also as to Q-factor.

If this segment were composed of a single homogeneous material, a low frequency alternating current flowing therethrough would distribute itself evenly. Should it be shown that the current distributes itself unevenly, it would follow that the segment in question is heterogeneous as to impedance. The tissues of low impedance, in this case, would show the largest current densities and those of high impedance, the smallest. Two methods for measuring current density in a body segment are given below.

Flux Density Method. The experimental principle employed in this method is the following: The arms, carrying pure sinusoidal alternating current, are treated as the primary of a transformer and variations in current density are determined by moving a small exploring coil, acting as a secondary, over the arm surface. The current induced in the exploring coil is then amplified and shown in a proper indicating instrument.

The circumference of the arms was explored in this way at 3 levels: (1) in a transverse plane passing through the elbow, (2) in a similar plane passing through the arm midway between elbow and axilla and (3) in a plane passing through the axilla and the outer border of the acromion. In addition, the thorax was explored at the various points on its anterior and posterior surfaces to determine the upper and lower limits of the current path.

Experimental Procedure: The source of current used was a cathode-ray type phasemeter (Radio Instruments Co., model G) having a built-in V-T voltmeter and supplying substantially harmonic-free oscillations at a frequency of 11,000 cycles and 0.3 volt. The exploring coil was wound in the form of an annulus of square section one inch in diameter composed of 500 turns of No. 40 wire and had a total impedance of 290 ohms at the frequency employed. It was completely shielded electrostatically and also shielded electromagnetically on all sides except for a small opening exposing the coil to the magnetic field of the body. The current induced in the exploring coil was fed into a variable gain 120 db. A-F amplifier con-

nected on its output side to the V-T voltmeter of the phasemeter. All leads were shielded and made long enough to permit measurements to be made 8 feet from the phasemeter and well outside any field generated therein. The indications of the V-T voltmeter appeared on the cathode-ray tube screen as a horizontal band whose width varied with the intensity of the field cutting the exploring coil. A scale graduated in one-half inch divisions mounted in front of the screen permitted measurement of the band width and could be read with magnification to 0.1 division. Current density measurements were made with the subject standing and the arms in a horizontal position. Absorbent cotton soaked in 1% saline was wound around the forearm to a point about 5 inches below the elbow level and held in place by a spiral winding of one inch lead tape. Current was fed to the arms by means of 8-foot leads attached to the lead tape windings.

In making measurements, the small opening in the electromagnetic shielding surrounding the exploring coil was brought into apposition with the surface of the arm (grounded side) or the chest so as to expose the coil windings to the field of the body and the coil was then slowly rotated until the band on the cathode-ray screen gave a maximum width. This was found to occur when the coil lay in a plane passing through the longitudinal axis of the arm and in a horizontal plane passing through the thorax. Under these conditions, the coil was being cut by the maximum flux in the underlying tissues.

Preliminary tests having shown that the results obtained were qualitatively the same for different individuals, final measurements were made on a lean normal female, 26 years of age, having an arm circumference at the mid-biceps level of 8 inches.

The form of the fields† around the right arm is shown in Fig. 1.

The outline of the field shows the current density to be a minimum over the olecranon and the acromion and to increase moderately over the larger muscles to reach a maximum at the bend of the elbow, on the medial side of the upper arm and just behind the anterior wall of the axilla, *i. e.,* following the neuro-vascular bundle containing the median nerve. The small difference in current density for points over the olecranon and over the ulnar nerve is noteworthy.

Exploration of the thorax showed the upper and lower limits of the current path to lie between 2 horizontal planes passing through

† The fields shown represent the average of 2 independent measurements made with the current leads and phasemeter placed first in front of, and then behind, the subject. This was done to correct for small effects on the magnetic field of the body due to the position of the leads themselves.

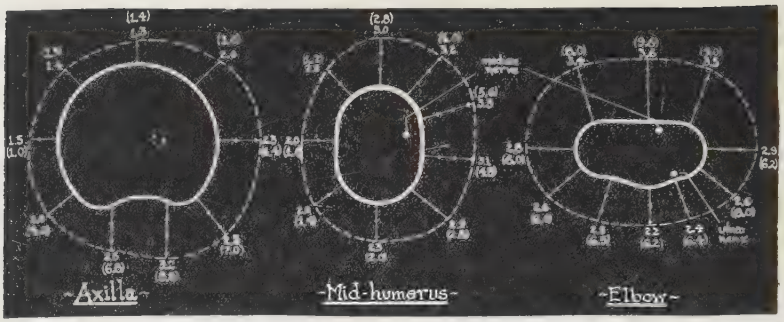

FIG. 1.

Current densities measured at the surface of the arm. The 3 sections are taken in transverse planes passing through the axilla, mid-humerus and elbow of the right arm. The form of the magnetic field around the arm is shown in dotted lines. The flux densities indicated are in arbitrary units.

Changes in phase angle obtained by the capacity increment method are shown in parenthesis (observed values multiplied by 1000).

the 2 axillae and through the base of the neck. The field was found to diminish in a regular way from values shown in the section through the axilla (Fig. 1) to a value of 1.0 division as the exploring coil was moved inwardly along the anterior and posterior chest wells from the shoulders toward the sternum and spine. Repeated explorations over the anterior surface of the neck failed to show any increase in field strength in the region over the thyroid gland.

Constant Capacity Increment Method. This method for measuring current density was designed to be independent of any magnetic field due to the current leads and to serve as a check on the results obtained by the flux density method. The experimental principle involved is the following: If the Q-factor of the arm-to-arm segment be measured first by one of the usual methods (immersion or 4-electrode technic) and then with a strip of metal applied over a given skin area, the second reading will be increased over the first by an amount dependent on (1) the capacity of the skin lying beneath the metal strip and (2) the local current density in the same area. The skin, under these conditions, acts as a dielectric enclosed between the metal strip and the deep tissues as conducting plates. Since the capacity per unit area of the skin in the region of the arms and chest may be assumed to remain substantially constant, a series of measurements with the metal strip applied over the various areas permits a mapping of the local current densities below the skin surface. Measurements of current density were made by this electrostatic method over the areas indicated in Fig. 1 which had been previously explored electromagnetically.

Experimental Procedure: The subject was instructed to immerse

the forearms in 2 arm baths each containing 12 liters of one percent saline, palms down on the arm bath bottoms and the forearms extending upward substantially vertically. A Q-factor measurement was then made in the usual way with the cathode-ray phasemeter. A strip of tin 4″x1″x0.01″ encased in a closely fitting flannel cover and soaked in one percent saline was then applied by hand (a rubber glove served to insulate the hand from the strip) to various portions of the arm and chest with sufficient pressure to insure good contact, the length of the strip being maintained parallel to the direction of flow of current from arm to arm. The increase in Q-factor could then be measured with the phasemeter. In making measurements in the axilla and at the elbow, the strip was bent to conform to the skin surfaces in these regions.

The results obtained by this method are in good qualitative agreement with measurements of flux density. The Q-factor values remained unmodified with the strip applied over the anterior and posterior chest surfaces as well as over the thyroid gland. Along the arms and in the axilla, the changes indicated in parenthesis in Fig. 1 were obtained. The values given represent the Q-factor change multiplied by 1000. Thus (5.0) is equivalent to a change of 0.0050.

At the elbow level the capacity increment values found are somewhat higher than would be expected from the flux density results. This would appear to be due to the fact that measurements of flux density represent the additive effect of the flux in a group of parallel current paths taken through a considerable portion of the arm thickness, whereas those obtained by the capacity increment method correspond to conditions immediately beneath the skin surface. It is to be noted, however, that the relative values obtained by both methods in passing around the arm at any given level show the same general type of current distribution.

The concordant results obtained by the flux density and constant capacity increment methods which are based upon independent electromagnetic and electrostatic modes of exploration leave little doubt that the arm-to-arm segment is best represented as a heterogeneous impedance, and it remains now to be shown that the segment in question is also heterogeneous as to Q-factor. To do this, it is only necessary to compare the Q-factors of the constituent portions of this segment which is composed of (1) a chest portion or section containing a large mass of pulmonary tissue, (2) a shoulder portion traversed by tendinous and muscular masses and (3) an arm portion proper consisting predominantly of muscular tissue. Each of these portions or sections differs markedly in size, shape and

internal structure from the others. It will be recalled that, in the 4-electrode method of Horton and Van Ravenswaay, current is passed from arm to arm by means of a first pair of electrodes and the (inner) impedance properties of any portion of the body lying between these current carrying electrodes is measured by tapping off voltages from a second pair of spaced bands. If this pair of voltage tapping bands be placed over each of the shoulders and under the corresponding axilla, the Q-factor of the chest section so delimited may be measured separately. A similar pair of spaced bands mounted on the upper arm permits a measurement of the upper arm alone, while with one band on the upper arm and the other passing under one axilla and over the corresponding shoulder, values for a shoulder section may be obtained.

Experimental Procedure: The original method of Horton and Van Ravenswaay was employed with the following minor modifications: Current was fed to the arms *via* a pair of arm-baths containing 1% saline. The voltage tapping bands used by these authors (phosphor-bronze) were found to be too stiff to lie in contact with the axillae and were replaced by ordinary solder wire 1/16″ in diameter, the skin being moistened with normal saline before applying the wire thereto. Measurements of Q and Z were made by tapping off voltages from the bands to the grids of 2 tubes and determining (1) the amount of resistance necessary to produce an equivalent voltage drop and (2) the difference in phase between the current and voltage, these values being obtained by means of a special circuit (to be described elsewhere), coupled to the cathode-ray phasemeter already mentioned and functioning as an a.c. comparator.

The Q-factors of these 3 separate body sections in 6 unselected normal subjects of varying physical conformation are given in Table I. Differences in physical conformation are indicated by the arm circumferences and the elbow-to-elbow length. It will be seen

TABLE I.
The Q-factor of Component Sections of the Arm-to-arm Segment.

Subject	Sex	Age	Arm circumference in cm.	Elbow-to-elbow length in cm.	Q-factor		
					Arm Section	Shoulder Section	Chest Section
1.	Female	37	26	90	.047	.066	.056
2.	”	26	28	89	.060	.079	.071
3.	Male	20	22	87	.063	.108	.082
4.	”	19	30	96	.099	.106	.081
5.	”	23	28	102	.065	.116	.092
6.	”	48	24	90	.068	.086	.056

that the Q-factor of these various sections may vary among themselves by ratios as great as 1.8:1, the value obtained for any particular section depending, apparently, on its size, shape and internal structure.

Summary and Conclusions. The arm-to-arm segment is shown to be heterogeneous both as to impedance and Q-factor. It follows, therefore, that the Q-factor of this segment cannot properly be assumed to be independent of differences in physical conformation. Q-factor measurements which fail to take into account the possible effect of size and shape are open to serious question.[5]

9302 P

High Intestinal Obstruction In the Dog Treated with Extract of Adrenal Cortex.

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(Introduced by John A. Kolmer.)
From Temple University Medical School.

Acute intestinal obstruction as a representative of a group of conditions that are characterized by hyperazotemia and hypochloremia bears resemblance to adrenal insufficiency.[1, 2, 3] Indeed, we described the histologic[1] changes of the adrenal glands in the dog with high intestinal obstruction as that of lipoid exhaustion of extreme degeneration of the adrenal cortex.*

In view of Kendal's[5] separation of 2 fractions of the cortical extract one of which produces results only when salt is administered with it, it seemed desirable to study the combined effect of cortical

5 Horton, J. W., and Hertz, S., *Endocrinology*, 1936, **20**, 831.

1 Wohl, M. G., Burns, J. C., and Clark, J. H., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 543, 546.

2 Wohl, M. G., and Brust, R., *J. of Lab. and Clin. Med.*, 1935, **20**, 1170.

3 McCance, R. A., *Lancet*, April 11, 1936, page 829.

* Dr. J. E. Sweet called the attention of one of our coworkers, Dr. J. H. Clark, to the fact that in several of his writings he spoke of the peculiar similarity between dogs that have died after bilateral suprarenalectomy and those from toxic injection.[4] We are glad to make this correction. However, Dr. Sweet stresses the changes that occur in the medulla of the adrenals and not in the cortex which we are inclined to look upon as one of the chief factors concerned in the disturbed pattern of the water and electrolyte metabolism in high intestinal obstruction.

4 Sweet, J. E., *Penna. Med. J.*, April, 1913.

5 Kendal, E. C., *J. A. M. A.*, 1935, **105**, 1486.

extract and salt solution in high intestinal obstruction. The amount of salt solution employed was considerably less than previously found sufficient to prevent adrenal exhaustion.

High intestinal obstruction was induced on 6 dogs. The method, the preoperative and postoperative care were the same, as employed previously.[1] Daily blood studies were made for NaCl, urea nitrogen, CO_2 combining power of blood plasma, hemoglobin specific gravity and red blood cell count.

Two dogs received 200 cc. of physiological salt solution intraperitoneally on the day of operation and every day thereafter plus 3 cc. of adrenal cortex extract intramuscularly; 2 dogs had neither cortin, nor salt solution; 2 dogs received daily intramuscular injections of 3 cc. of extract of adrenal cortex.† The dogs receiving adrenal cortex and salt solution lived for 15 days, when they were anesthetized and killed. They showed a loss of weight of 1.3 kilos and towards the last 2 days developed twitchings of muscles of hind legs. The blood urea nitrogen rose from 9.8 mg. % on the day of operation to 21 mg. % on the day of postmortem. The blood chlorides decreased from 510 mg. % to 420 mg. %. The CO_2 increased from an initial 48.5 volume % to 75.3 volume %. The specific gravity remained practically the same. (1.028 to 1.030). The hemoglobin decreased from 14 gm. to 10.5 gm. The red blood cells decreased by 1,470,000. The postmortem and histological findings of the organs as well as those in other dogs will be described elsewhere.[6] The dogs without salt solution and cortin lived 5 days. They became toxic; vomited on the second day after operation, gradually grew worse and died on the fifth day after operation. The blood urea nitrogen increased from 16.1 mg. % on the day of operation to 144.2 mg. % on the day of death. The blood chlorides decreased from 440 mg. % to 302 mg. %. The specific gravity of the blood increased from 1.025 to 1.043. The hemoglobin has increased from 8.5 gm. to 17 gm. The red blood cells increased by 1,410,000.

The dogs receiving cortin alone lived for 7 days. They lost one kilogram in weight; they apparently were not toxic and on the seventh day developed twitchings of muscles of trunk and of all 4 extremities and died. On this day, an additional dose of 3 cc. of cortin was given without much benefit. The blood changes were as follows: The blood urea nitrogen increased from an initial 14.7 mg. % to 66.5 mg. % on the day of their death; the blood chlorides de-

† We are indebted to Dr. David Klein of Wilson Laboratories, Chicago, Ill., for the supply of adrenal cortex extract.

[6] Wohl, M. G., Clark, J. H., and Burns, J. To be published.

creased from 480 mg. % to 260 mg. %. The CO_2 increased from 48.1 vol. % to 78.2 vol. %. The specific gravity increased from 1.027 to 1.030; the hemoglobin decreased from 14 gm. to 11 gm., and the red blood cells decreased by 1,300,000.

Heuer and Andrus[7] prolonged the lives of dogs receiving intravenous injections of aqueous extracts of closed intestinal loops by administering cortical extract combined with transfusion. Scudder, Zwemer and Truszkowski[8] fortified our comparison between the clinical and biological picture of acute intestinal obstruction and adrenal insufficiency by demonstrating high blood potassium values in cats with high intestinal obstruction. This assumes special importance in view of Zwemer's and Truszkowski's[9] findings that symptoms of adrenal insufficiency may be explained in terms of a disturbance of cortico-adrenal-potassium interrelation.

We appreciate that our experiments are too few in number to permit the drawing of definite conclusions, but one would seem justified in emphasizing: 1. The many features in common in acute intestinal obstruction and adrenal insufficiency. 2. The apparent benefit from the combined administration of physiological salt solution and adrenal cortex extracts in combating the toxemia of high intestinal obstruction.

9303

Diffusion of Ions Through Collodion Membranes Treated with Urethanes.

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From the Biological Laboratory, Cold Spring Harbor.

Anselmino[1] has shown that collodion membranes, made so as to permit a slow passage of thiocyanate ion, have a lowered permeability to this ion when the dialysis system is treated with urethane, and that after the "narcotized" membrane is washed for about an hour with water, the normal thiocyanate permeability is restored. This observation has been frequently used in connection with the thesis that narcotics produce their effects through changes in per-

7 Heuer, G. J., and Andrus, W. D., *Ann. Surg.*, 1934, **100**, 734.

8 Scudder, J., Zwemer, R. L., and Truszkowski, R., *Surgery*, St. Louis, 1937, **1**, 74.

9 Truszkowski, R., and Zwemer, R. L., *Biochem. J.*, 1936, **30**, 1345.

1 Anselmino, K. S., *Pfluger's Arch.*, 1928, **220**, 524.

meability. When the experiments were extended to the study of the Cl ion, however, similar but less convincing results were obtained; we have, therefore, felt that a more complete investigation of the problem was necessary.

Collodion membranes were made as described by Anselmino, and 5 cc. of M/2 KSCN placed on one side, and distilled water on the other. Determinations of the amount of thiocyanate passing were made at 3, 6, . . . 15 minute intervals by the addition of ferric chloride and comparison with standards. The experiment was then repeated with M ethyl carbamate on both sides of the membrane, and the retardation of diffusion described by Anselmino was confirmed, even when the ionic strengths on both sides of the membrane were equalized by the addition of KCl. Propyl, butyl, iso-amyl, and phenyl carbamate produce a similar effect, the concentration required decreasing with the length of the carbon chain.

On extending the investigation to the rates of diffusion of other ions, however, we found the rates to be quite unaffected by the presence of any of the carbamates, for chloride ion (silver precipitation determination), sulphate ion (Ba determination), nitrate ion (phenylsulphonic acid determination), iodide ion (S_2O_3 determination), permanganate ion, ferrocyanide and ferricyanide ions ($FeCl_3$ determination), ferrous and ferric ions, and finally potassium ion (cobaltinitrite determination) all pass through the collodion membranes as rapidly in the presence of the carbamates as in their absence. It therefore seems that the effect of the narcotics is a specific one for thiocyanate ion, and does not have the general significance which has been attributed to it.

Collodion membranes containing lecithin were then prepared, and when these were used in the same type of experiment we found the addition of the carbamates to cause an *acceleration* of the diffusion of thiocyanate ion, instead of an inhibition. This effect depends on the quantity of lecithin present in the membrane (Table I).

The narcotics, however, produced no effect whatever on the diffusion of sulphate, permanganate, or ferricyanide ion through these

TABLE I.

p. c. lecithin per gm. dry membrane	Ethyl carbamate	mg. SCN' penetrating in 15 min.
.01	Absent	8.
	0.5 M	12.
.05	Absent	6.
	0.5 M	12.
.10	Absent	9.
	0.5 M	25.

lipoid-containing membranes, and again we seem to be dealing with some effect specific for the thiocyanate ion.

In the case of collodion membranes containing varying amounts of lecithin, from 0.01 to 0.1 p.c. of lecithin per gm. of dry membrane, together with varying amounts of cholesterol, from 0.01 to 0.1 p.c. per gm. of dry membrane, the carbamates again produce an acceleration of the diffusion rate of thiocyanate, but the effect becomes less as the amount of cholesterol is increased. The passage of the other ions studied was not affected by the addition of the narcotics.

9304 P

Adrenalectomized-Depancreatized Dogs.

C. N. H. LONG, F. D. W. LUKENS AND F. C. DOHAN.

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

We have previously reported[1] that removal of the adrenal glands from the cat ameliorates the effects of pancreatic diabetes to an extent comparable to that obtained by hypophysectomy and, furthermore, that the removal of the adrenal cortex is responsible for this effect of adrenalectomy. Houssay and Biasotti[2] have recently reported a similar effect of adrenalectomy upon pancreatic diabetes in the toad. Since the dog has been extensively used in the study of experimental diabetes and since several workers have reported that adrenalectomy does not modify an experimental diabetes[3, 4, 5] in this species, it was of some interest to prepare long surviving animals in which all adrenal and pancreatic tissue had been removed. This was accomplished by first removing one adrenal and at a later date, the pancreas. The animals were then maintained by use of protamine insulin until in good health, when the second adrenal was removed and the insulin discontinued. During the remainder of their lives, they received a diet of meat and raw pancreas and, in addition, from 4 to 8 gm. of NaCl daily. They also received 5-10 cc. of cortical extract (Upjohn) daily. Analyses of the blood at

1 Long, C. N. H., and Lukens, F. D. W., *J. Exp. Med.*, 1936, **63**, 465.

2 Houssay, B. A., and Biasotti, A., *Rev. Soc. Argent. Biol.*, 1936, **12**, 104.

3 Stewart, G. N., and Rogoff, J. M., *Am. J. Physiol.*, 1923, **65**, 319.

4 Turcatti, E. S., *Rev. Soc. Argent. Biol.*, 1929, **5**, 173.

5 Leloir, L. F., *Rev. Soc. Argent. Biol.*, 1934, **10**, 216.

the end of the experiment indicated that no gross disturbances in the electrolyte balance had occurred. In consequence, it is assumed that the alterations in the character of the diabetes were not to be attributed to a distorted water and salt metabolism.

Table I shows that the glucose, nitrogen and acetone excretion of the 5 dogs studied was strikingly reduced compared to the quantities reported by Chaikoff[6] in fasting depancreatized dogs from which

TABLE I.

Dog No.	Survival days	Period of Observation days	Urine			Notes
			Glucose g./k./d.	Nitrogen g./k./d.	Acetone mg./k./d.	
		A. Adrenalectomized-Depancreatized Dogs.				
16	32	6	0.2	0.7	0	400 gm. meat and pancreas daily
17	21	3	0.9	0.8	0	150 gm. meat, 100 cc. milk daily
18	11	6	1.9	0.9	29	350 gm. meat daily
19	25	10	0.7	0.8	17	350 gm. meat daily
26	9	4	0.1	0.6	3	Fasted
		B. Fasting Depancreatized Dogs (Six).				
			2.9	0.8	187	After Chaikoff.[6]

insulin had been withdrawn. While we have no comparable figures for the survival of depancreatized dogs, it would appear that as in the cat, the length of life of the adrenalectomized-depancreatized dog is also appreciably extended. None of our dogs relapsed into acidosis or coma but slowly became free from glycosuria as their weight declined. This occurred even if the food intake remained constant. Hypoglycemic episodes relieved by glucose also were observed in one animal.

In 2 animals the ketonuria for a short period was greater than we have observed in adrenalectomized-depancreatized cats (40-70 mg. a kilo a day) but this appears to be a species difference as hypophysectomized-depancreatized dogs exhibit a greater ketonuria than do similarly operated cats.

It is therefore concluded that adrenalectomy produces the same modification of pancreatic diabetes in the dog as has already been observed in the cat.

[6] Chaikoff, I. L., *J. Biol. Chem.*, 1927, **79**, 203.

9305 P

On the Site of the Formation of Citric Acid in the Animal Body.

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Recent studies[1, 2] have demonstrated that the intravenous injection into dogs of the sodium salts of certain dicarboxylic acids, including malic acid, produces a marked and prompt increase in the amount of citric acid excreted in the urine. Simultaneous determinations of the citric acid content of urine and blood[2] suggested that the kidney played an important part in the formation or elimination of citric acid after the injection of sodium malate. The following experiments were designed to more definitely elucidate the question of the site of citric acid formation under these experimental conditions.

Adult male rats weighing from 250 to 450 gm. were given a citrate-low synthetic ration (casein, 18%; dextrin, 51%; Crisco, 27%; Wesson salt mixture, 4%) for a 5-day preliminary period. The animals were then lightly anesthetized with ether and sterile solutions of either sodium chloride (2.5%) or sodium malate (5%) were injected into the heart in amounts containing 100 mg. of sodium per kilo body weight. Preliminary studies showed that, under these conditions, the rat, like the dog, excretes relatively large amounts of citric acid in the urine after the parenteral administration of sodium malate. In the present experiments, the animals were again anesthetized 15 minutes after the injection of the chloride or malate, exsanguinated by bleeding from the abdominal aorta, and the tissues to be analyzed were removed and quickly frozen with carbon dioxide ice. The tissues were then pulverized, extracted with dilute sulfuric acid, and the citric acid content of aliquots of the protein-free (trichloroacetic acid) filtrates was determined. The amount of citric acid in trichloroacetic acid filtrates of the oxalated blood was also determined.

Table I shows that there was little difference between the citric acid content of the liver, muscle, and blood of the chloride-injected control rats and that of the malate-injected animals, whereas the citric acid content of the kidneys of the latter group was approximately twice that of the controls.

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* Chas. Pfizer Co., Inc., Research Fellow, Yale University, 1936-37.

1 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1937, **117**, 555.

2 Orten, J. M., and Smith, A. H., Presented before Am. Soc. Biol. Chemists, Memphis, Tenn., April 23, 1937. Proceedings, p. lxxiv.

TABLE I.
Citric Acid Content of Tissues.*
(Expressed as mg. %.)

	Intact Rats				Nephrectomized Rats			
	Sodium Chloride Injected		Sodium Malate Injected		Sodium Chloride Injected		Sodium Malate Injected	
Tissue	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
Liver	1.6	1.0-2.2	1.4	1.2-1.7	2.5	1.7-3.1	2.3	1.4-2.7
Kidney	5.5	3.0-7.0	11.7	8.0-16.1	—	— —	—	— —
Muscle	2.7	1.7-3.7	2.6	1.5-2.9	4.2	2.9-5.2	3.3	2.2-4.2
Blood	4.0	3.3-4.8	4.0	3.4-4.3	4.6	2.9-7.5	4.7	2.5-6.3

*Averages represent 5 rats in each group except those for kidneys of intact rats, where 8 animals were used.

In order to determine whether the "extra" citric acid present in the kidney after malate injection may have been formed elsewhere and was merely being cleared from the blood by the kidneys, further studies were made on a second series of rats treated in the same manner as those just described, but which were bilaterally nephrectomized immediately preceding the injection. The table shows that again there was little difference between the citric acid content of the blood, liver, and muscle of the control and malate-injected animals.

Thus it is evident that under the conditions employed, the citric acid formed following the injection of sodium malate into rats is produced chiefly, if not entirely, in the kidney.

9306

On Factors Limiting Bacterial Growth. I.

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There is as yet no agreement among bacteriologists as to the specific physical and chemical influences responsible for changes observed in the rate of growth during the life of the bacterial culture.[1] In Fig. 1 typical curves are presented, illustrating these changes as they occurred during the development of a broth culture of *Bacterium coli.* The plotted values were calculated by us from the data of Martin,[2] by means of the formula B-A/T for the rate of population-increase,

[1] Topley and Wilson, *The Principles of Bacteriology and Immunity*, 1929, **1**, 75.
[2] Martin, D., *J. Gen. Physiol.*, 1931, **15**, 697.

and log B-log A/T log 2 for the rate of growth, where A represents the population at the beginning and B at the end of the observed time-interval T. It will be seen that during the period of rapid increase in population, the rate of growth shows a sharp rise, followed by a continuous decrease. It has been the purpose of our experiments to reinvestigate the causes for these changes.

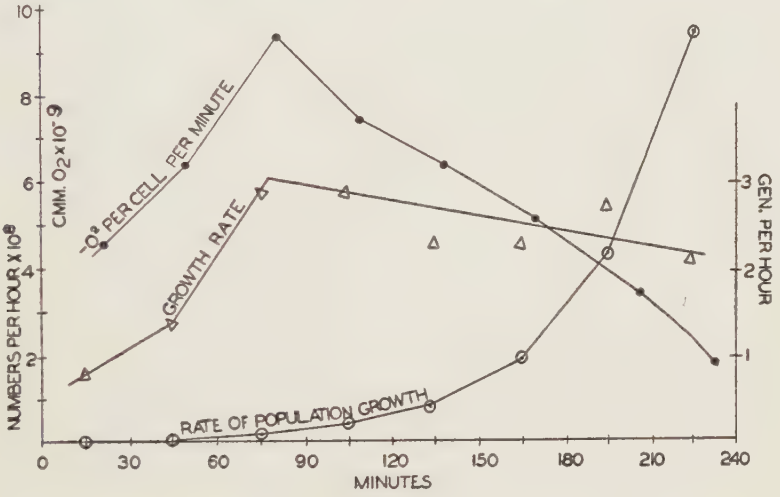

Fig. 1.

Rates of growth and oxygen-uptake per cell during period of increase of a broth culture of *Bact. coli*. (From data of Martin.[2])

The possibility had presented itself that measurements of rate of oxygen-consumption per cell might be used as a convenient index of comparative growth-rates. The observations of Martin on the oxygen-uptake of *Bact. coli* cultures seemed to support this inference. In Fig. 1 we have plotted the rates of oxygen-consumption which he observed, using a single time-scale for these and for the corresponding growth-rates. A comparison of these curves shows that a close relation exists between the rate of growth, and the rate of oxygen-uptake per cell, throughout the duration of the experiment. The present paper deals with the influence of certain factors on the rate of oxygen-consumption per cell. Future publications will present fuller justification for extending conclusions based on these data to the question of actual growth-rates.

The methods we have used were essentially the same in all experiments. Bacterial cells to be examined were centrifuged from the culture fluid, resuspended in sterile salt solution, and standardized both by photoelectric estimation of turbidity using solutions of copper sulfate as standard for comparison, and by the dilution and pour-

plate method to obtain the viable count. The medium used was ordinary 0.5% beef extract—1.0% peptone broth, containing sufficient phosphate buffer of pH 6.6 to give a final concentration of M/20. Whenever the culture-fluids were to be examined, they were sterilized by heat or by filtration, with or without readjustment of pH. The required amount of suspension, buffer, and saline were pipetted onto the floor of the respirometer-vessel, and peptone broth of 5 times the desired concentration was placed in a side arm. When necessary, as in the examination of culture-filtrates, this procedure was reversed, the cells being placed in the side arm and the culture-fluid in the vessel proper. Rolls of starch-free filter paper moistened with 10% KOH served to adsorb the carbon-dioxide liberated. The vessel was then attached to its differential manometer of the Barcroft type, together with a compensator containing the same amounts of nutrient broth, and the apparatus was allowed to reach equilibrium in a waterbath held at 37 ± .001°C., by means of a thyratron-relay. Manometric readings were begun and continued at 5-minute intervals following the introduction of the substrate from the side-arm. With the corrected values of average oxygen-uptake for the successive 5-minute intervals, curves were laid down, and extrapolated graphically to the vertical axis, where the hypothetical quantity of oxygen consumed per 5 minutes at zero time could be read off. In this way it was possible to obtain consistent values for oxygen-uptake per cell to the exclusion of complicating factors of cellular multiplication and changing composition of medium.

It is evident that changes in the rate of growth may be dependent either on the physiology of the cell, on the nature of its environment, or on both. Our first experiments were directed toward separate study of changes in cellular activity, and alterations in the medium of growth in relation to the multiplication-rate observed in broth cultures of *Bact. coli*. The cultures for examination were prepared by inoculation of 100 ml. of infusion-broth in 500 ml. Erlenmeyer flasks with 0.1 ml. of an 18-hour test-tube culture in the same medium and incubated at 37.5°C. without resort to special methods of aëration. The results obtained by the methods outlined above may be summarized very briefly for our present purpose by saying that neither the changes detected in the rate of oxygen-uptake of cells taken from cultures of different age, nor differences in the effect of the corresponding culture-filtrates upon growth and respiration, were sufficiently great to explain the divergence of growth-rates observed at different times in the parent-cultures. Some additional environmental factor apparently was involved.

The remaining factor, which in the above experiments had been

kept constant, was the density of population of the test-suspension. By adaptation of the methods already used, we next measured the oxygen-consumption per cell at zero time in nutrient broth into which a standard amount of bacterial suspensions of varying density were introduced. The results of these tests with 2 and 5 ml. volumes of culture-suspension respectively are shown in Fig. 2.

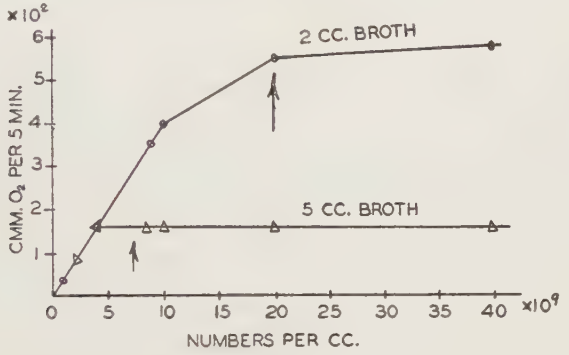

Fig. 2.

Rates of O_2-uptake at zero time per ml. of 2 and 5 ml. broth cultures seeded with different numbers of *Bact. coli*.

Arrows indicate maximal numbers attained in cultures growing under the respective conditions from small inocula.

The first observation of interest is that with equal surface-area and with populations in excess of 10^9 cells per ml., the volume of the culture has a marked influence upon the energy available per unit-volume of the medium. The difference in oxygen-uptake per ml. between the 2 and 5 ml. cultures respectively could be attributed to the limited amount of oxygen which can dissolve in the medium through a given surface, thus giving advantage to cells in the culture where the surface-volume ratio was larger. There is nothing surprising in this finding, which we report only because it seems to imply that nearly all quantitative studies of bacterial metabolism reported in the literature have been carried out under conditions where the available supply of oxygen was limited, even when forced aëration was maintained. It should be noted that the curve showing oxygen-starvation in the 5 ml. culture was obtained under conditions in which the depth of the medium exposed to the air was only 2 mm., and which were shaken in the usual manner 78 times per minute through an amplitude of 5 cm., while with 2 ml. cultures, having a depth of about 0.8 mm., we have not seen any indication of oxygen-insufficiency.

The second fact of interest shown by these curves is that the

limiting density of population in test-suspensions which permitted the rate of oxygen-consumption per cell to remain at its optimum in the 2 cases, was very nearly the same as that reached in normal cultures at the time when active multiplication ceased under the corresponding conditions. These points are indicated in Fig. 2 by arrows. This correlation suggests that analysis of factors limiting oxygen-consumption per cell will, in fact, explain cessation of growth.

The evidence that in cultures having a total volume of 5 ml., under the conditions of our experiments, in which the density of population approaches 10^9 cells per ml., the oxygen-uptake per cell is limited solely by oxygen-supply, may be summarized as follows:

(1) Increasing the surface-volume ratio from 5 to 12.5 by decreasing the volume from 5 to 2 ml., increased the oxygen-uptake per cell; whereas increasing this ratio from 12.5 to 25 by further reducing the volume to 1.0 ml., left oxygen-uptake per cell unaffected. (2) Increasing the rate of shaking increased the oxygen-uptake per cell in 5 ml. cultures, but not in 2 ml. cultures. (3) Increasing the partial pressure of atmospheric oxygen markedly increased this value in 5 ml. cultures, but very little in 2 ml. cultures.

It is clear, therefore, that under ordinary conditions of cultivation in stationary cultures, where the surface-volume ratio varies from about 0.2 in test-tube cultures to somewhat more or less in flasks of different shape containing the same depth of medium, the rates of solution and diffusion of oxygen will be of critical importance in the economy of the culture. We are at present pursuing the question of the extent to which oxygen-deprivation limits the rate of growth and the maximal population attained under various conditions of aëration.

We have indicated that in the 2 ml. cultures used in the respirometer-experiments, oxygen was available in excess of the demand within the range of density of population employed. What is it then that limits the respiration and growth in these cultures? It was noted that shortly after the maximal value was attained under these conditions, the rate of oxygen-uptake of the culture rapidly decreased to values approaching zero, whereas in 5 ml. cultures it remained constant at its maximum during several hours. If cells from the apparently lifeless 2 ml. culture were transferred to fresh medium, they took up oxygen and multiplied normally. The pH of the original culture had remained within physiologic limits. It appeared, therefore, that under these conditions important changes in the composition of the culture-fluid had occurred, although no such changes had been found in earlier experiments employing fil-

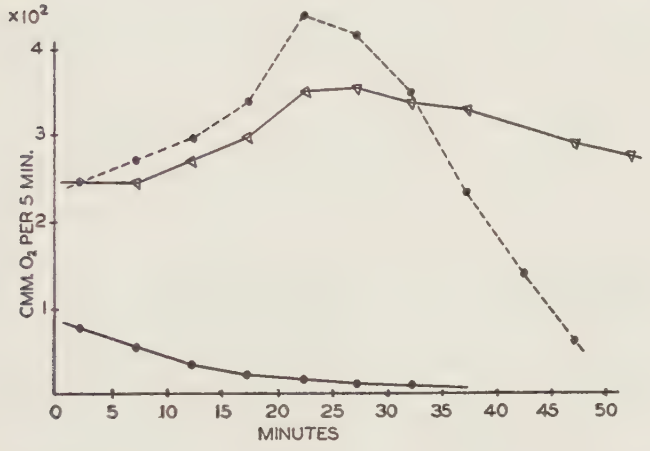

Fig. 3.

Rates of O_2-uptake of 2.2 x 10^9 *Bact. coli* in media containing filtrate of an aërated broth culture.

-●------ 1.5 ml. 1% peptone + 0.5 ml. saline.
-△—△— 1.5 ml. filtrate + 0.5 ml. 3% peptone.
-●——●— 1.5 ml. filtrate + 0.5 ml. saline.

trates of cultures maintained under more usual conditions. In Fig. 3 we have shown data obtained by reseeding the sterile filtrate obtained at the end of the growth-cycle from an intensely aërated culture. It may be seen that this filtrate does not support the respiration of *Bact. coli,* but that its usefulness is largely restored by the addition of 1% peptone. These findings suggest that growth of *Bact. coli* ceases under these conditions as a consequence of oxidation of essential available food materials. So far our experiments have not lent support to the commonly accepted view that accumulation of growth-inhibitory metabolic products is responsible for cessation of growth in bacterial cultures. It appears that under the usual conditions of cultivation, rates of growth and respiration of *Bact. coli* are limited by the rate at which oxygen can reach the cells, and that this limitation prevents rapid exhaustion of the nutrient materials. If, however, oxygen is available in excess, growth soon ceases as a result of oxidative removal of foodstuffs.

9307 P

pH Stability of Shope Papilloma Virus and of Purified Papilloma Virus Protein.

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We described[1] the isolation of a high molecular weight protein from virus-induced papillomas of western cotton-tail rabbits.[2] It was found that this protein could be extracted only from infectious suspensions of such papillomas and that virus activity was proportional to the amount of the protein present. This and other experiments, in which we have failed to dissociate virus activity from the protein, point to its being the purified agent responsible for the disease.

We have now completed a detailed comparison of the influence of pH on (1) the infectiousness of the virus and (2) the stability of the molecules of the protein. Stability of the virus was determined by dissolving the virus protein in buffer solutions of accurately determined pH and titrating the solutions in domestic rabbits[3] immediately, and after 6 hours, 24 hours, a week and a month. With the analytical air-driven ultracentrifuge,[4] the molecular constitution of these solutions was found from their sedimentation diagrams.

On the acid side of pH 7 the virus activity remains high until at a pH between 2.9 and 3.3 it suddenly is lost. The protein molecule splits at exactly this point. From pH 3.3 to pH 7, on both sides of its isoelectric point, the papilloma protein has a principal component with $S_{20^\circ} =$ ca 260×10^{-13} cm. sec.$^{-1}$ dynes^{-1}; there ordinarily appears also a faint secondary boundary corresponding to $S_{20^\circ} =$ ca 380×10^{-13}. At pH 1.85 the completely inactive protein sediments with the sharp boundary corresponding to a single molecular species with $S_{20^\circ} =$ ca 180×10^{-13}.

In contrast to conditions in acid solutions, full virus activity is not preserved for a long time at any pH greater than 7. Alkaline inactivation occurs in two ways. Above pH 10.1 virus solutions immediately become non-infectious. Below this point the titre of a virus solution gradually diminishes with time, at a rate that decreases

1 Beard, J. W., and Wyckoff, R. W. G., *Science*, 1937, **85**, 201.

2 Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

3 Kidd, John G., Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1936, **64**, 63.

4 Biscoe, J., Pickels, E. G., and Wyckoff, R. W. G., *J. Exp. Med.*, 1936, **64**, 39; Wyckoff, R. W. G., and Lagsdin, J. B., *Rev. Sci. Instruments*, 1937, **7**, 246.

as neutrality is approached. Study of the stability of the protein molecule with the analytical ultracentrifuge demonstrates that it fragments at the same pH (10.1-10.2) at which immediate inactivation is observed. This molecular disintegration is more complete than the one seen in strongly acid solutions; the largest piece of the original protein that can be photographed in strong alkali has a sedimentation constant of only $S_{20^\circ} = \text{ca } 30 \times 10^{-13}$.

Below pH 10 the sedimentation constant of the papilloma protein molecule is not measurably changed nor is the molecular homogeneity visibly diminished even when solutions are kept until all virus activity has been lost. These solutions thus contain non-infectious protein material consisting of only slightly altered papilloma protein molecules. The biological properties of such non-infectious derivatives of the papilloma virus protein are being studied.

9308 P

Static and Kinetic Conditioned Reactions.*

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Former experiments of Spiegel[1, 2] and his coworkers (Aronson,[3] Price[4] and Spiegel) indicated that labyrinthine impulses may reach the cerebral cortex.

In order to investigate what part the connections of the labyrinth with higher centers play in the perception of position and motion, the study of conditioned reactions seemed promising. A special position table was built allowing dogs to be brought into any desired position. The dogs were slowly rotated from a sloping position through the horizontal plane into another oblique position. When the horizontal plane was passed, an electric shock (unconditioned stimulus) was applied to a leg, during the motion in one direction only; the defense reaction and the change in respiration were recorded. First, only conditioned reactions upon the horizontal position appeared. Later the animals learned also to differentiate be-

* Aided by a grant of the National Research Council to E. S.

1 Spiegel, E., *J. Nerv. and Ment. Dis.*, 1932, **75**, 504.

2 Spiegel, E., *Arch. Neur. and Psych.*, 1934, **31**, 469.

3 Aronson, L., *J. Nerv. and Ment. Dis.*, 1933, **78**, 250.

4 Price, J., and Spiegel, E. In press.

tween the direction of the motion accompanied by the unconditioned stimulus in the horizontal position (up or downwards) and the motion in the opposite direction during which the unconditioned stimulus was omitted. Thus static as well as kinetic conditioned reactions could be developed. These reactions appeared in the majority of our observations before the horizontal position was reached; in other words, they were mainly anticipatory in nature. In these cases the first appearance of conditioned reactions could be observed without omitting the unconditioned stimulus. Conditioned reactions in exactly the horizontal position were, however, also observed. The inference that we have here to do with conditioned reactions is based upon the fact that these reactions did not exist before the animals were trained, and that they showed typical characteristics of conditioned reactions, such as inhibition by fortuitous external stimuli, or extinction after repeated application of the conditioned stimulus without reinforcement by the unconditioned one. The strength of these conditioned reactions may sometimes exceed that of the unconditioned defense reflexes.

The effect of various peripheral and central lesions (elimination of labyrinthine and other afferent impulses, destruction of cortical areas) upon these conditioned reactions will be reported later.

9309

Sugar Alcohols VIII. The Oxidative Specificity of *Acetobacter Suboxydans.*

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Neuberg and Hoffmann[1] have observed that cultures of *Acetobacter suboxydans* in killed yeast medium containing 1% of glycerin will quantitatively oxidize the glycerin to dihydroxyacetone. In our studies[2] on the relationship between chemical constitution and utilization by bacteria of the sugar alcohols we became interested in determining whether the *Acetobacter suboxydans* oxidized specifically the secondary alcohol group in glycerin or, if its oxidative power was general for secondary alcohols.

[1] Neuberg, C., and Hoffmann, E., *Biochem. Z.*, 1935, **279**, 318.

[2] Dozois, K. P., Hachtel, F., Carr, C. J., and Krantz, J. C., Jr., *J. Bact.*, 1935, **30**, 190.

To a wide evaporating dish containing 500 cc. of boiling water 400 gm. of fresh brewers' yeast were added. The yeast was thoroughly ground with water until a smooth, thick paste-like mass was obtained. The mass was then transferred to a 3-liter flask and sufficient boiling water was added to make the total 2000 cc. The mixture was then boiled for 20 minutes, cooled to room temperature and placed in an ice-box for 48 hours. The supernatant fluid was siphoned off and filtered through paper until a clear, amber-colored liquid was obtained. This liquid was again boiled for 20 minutes, cooled and put in the ice-box for 48 hours and filtered. About 1200 cc. of the final product was obtained; this was placed in flasks, 200 cc. in each and sterilized in an autoclave at 15 pounds pressure. After sterilization the medium was stored at room temperature for 12 hours and the desired carbohydrate added to make a 1% solution. This medium was then inoculated with a culture of *Acetobacter suboxydans* and incubated at room temperature, in the dark for 7 days. The compounds studied were methyl alcohol, ethyl alcohol, propylene glycol, isopropyl alcohol, glycerin, trimethylene glycol, erythritol and mannitol.

Chemical tests for the products of oxidation of these compounds are well established and were employed to detect their presence or absence. None of the compounds studied was oxidized by the *Acetobacter suboxydans* culture except glycerin, which after a few days showed the capacity to reduce promptly Fehling's solution in the cold (dihydroxyacetone). It is surprising that substituting a methyl group for the primary alcohol grouping (CH_2OH) in glycerin with the formation of propylene glycol frustrates the capacity of the organism to oxidize the secondary alcohol group, which is common to each molecule, to a ketone. However, it has been demonstrated that the mycoderma aceti and sorbose bacteria[3] will oxidize propylene glycol to monohydroxyacetone. In addition, the placing of the secondary alcohol grouping between 2 methyl groups, as it appears in isopropyl alcohol, renders it refractory to the organism which cannot oxidize the compound to acetone. The secondary alcohol groupings of erythritol and mannitol, having a primary alcohol grouping attached to one adjacent carbon atom and another secondary alcohol grouping to the other, are likewise recalcitrant to the oxidative influence of the *Acetobacter suboxydans*.

Conclusion. From the compounds studied it is apparent that a secondary alcohol, to be oxidized to a ketone by the *Acetobacter suboxydans* by this procedure, requires a primary alcohol grouping

[3] Piloty, O., *Ber.*, 1897, **30**, 316.

on each adjacent carbon atom. The secondary alcohol group in glycerin only, meets these requirements; therefore, it is concluded that the *Acetobacter suboxydans* exhibits an oxidative specificity for the trihydric alcohol, glycerin.

9310 P

Correlation of *in vitro* Activity of Normal Human Gastric Juice on Casein at pH 7.4 with Gastric Intrinsic Factor.

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The administration of mixtures of normal human gastric juice (intrinsic factor) and beef muscle (extrinsic factor) at pH 5 or 7 to patients with pernicious anemia results in increased blood production and clinical improvement.[1, 2]

In 1930, in association with Dr. C. W. Heath, unsuccessful attempts were made to show that gastric juice at pH 7.4 caused the production of amino acid from beef muscle. Griffiths[3] showed that gastric juice incubated with beef muscle globulin at pH 6 produced certain chemical changes. Emerson and Helmer[4] could not confirm his results. However, the present observations show that when casein was substituted for beef muscle, gastric juice at pH 7.4 did produce progressive changes in the casein.

Normal human gastric juice was obtained free from bile after injection of histamine, filtered through gauze and placed in the ice box. A one percent solution of A. H. Thomas & Company washed casein was prepared at pH 7.4, avoiding excess of acid or alkali. To 50 ml. of this solution were added 50 ml. of normal human gastric juice at pH 7.4 and 2 ml. of toluol. The mixture was incubated at pH 7.4 for 24 hours at 37.5°C., the pH remaining essentially constant.

Ten ml. samples were removed at 4 hours and 24 hours for formol

[1] Castle, W. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sc.*, 1930, **180**, 305.

[2] Castle, W. B., *Science*, 1935, **82**, 159.

[3] Griffiths, W. J., *Biochem. J.*, 1934, **28**, 671.

[4] Emerson, C. P., and Helmer, O. M., *Am. J. Digestive Dis. and Nutrition*, 1936, **3**, 735.

titration. At the same time intervals 5 ml. samples were removed and the proteins precipitated with 20 ml. of 10% trichloracetic acid, and after standing at room temperature for 15 minutes, filtered through No. 12 Whatman folded filter paper. Total nitrogen determinations were made on the filtrates, using a micro-Kjeldahl digestion with subsequent distillation and nesslerization.

Eleven samples of normal gastric juice produced a progressive increase in a colloidal suspension in the filtrates which paralleled an increase in the total soluble nitrogen. This colloidal material could not be removed by centrifuging at 2500 r.p.m. There was, however, no corresponding increase in the amino nitrogen as determined by the formol titration of the digests.

Gastric juice was then subjected to certain procedures before mixing with the casein solution. The effect of the manipulations was compared with their known effect on the gastric intrinsic factor as shown by clinical observations in pernicious anemia. The intrinsic factor in gastric juice is not destroyed by passing through a Berkefeld V filter,[5] by exposure to pH 10 for 30 minutes at room temperature[6] or by removal of pepsin by isoelectric precipitation with casein at pH 4.7.[7, 8] Similarly when gastric juice was treated by these procedures its subsequent activity on casein at pH 7.4 *was not destroyed.*

When gastric juice is boiled for 5 minutes, heated at 70° to 80°C. for 30 minutes,[1] or incubated at pH 1.5 for 72 hours at 40°C. no subsequent hematopoietic activity with beef muscle appears clinically.[7] Likewise, as a result of such procedures, the activity of gastric juice on casein at pH 7.4 *was destroyed.*

Clinical observations have shown that saliva, pepsin U.S.P. and normal duodenal contents collected without admixture with gastric juice[1] are not sources of intrinsic factor. Saliva and pepsin solution had no action on casein at pH 7.4. A mixture of gastric juice and duodenal contents produced on incubation with casein at pH 7.4 increases both in total soluble nitrogen and in amino nitrogen as determined by formol titration. However, the increase in amino nitrogen was greatly reduced if the mixture was exposed to pH 10 for 2 hours at 37.5°C., although the mixture, like gastric juice after this treatment, retained its ability to cause an increase in total soluble nitrogen from casein at pH 7.4. On the other hand, 2 samples of

[5] Castle, W. B., Heath, C. W., Strauss, M. B., and Heinle, R. W., *Am. J. Med. Sc.*, in press.

[6] Flood, C., and West, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 542.

[7] Castle, W. B., Townsend, W. C., and Heath, C. W., *J. Clin. Invest.*, 1930, **9**, 2.

[8] Helmer, O. M., Fouts, P. J., and Zerfas, L. G., *Am. J. Med. Sc.*, 1934, **188**, 184.

gastric secretion obtained from patients with pernicious anemia and containing regurgitated duodenal contents showed an increase in the formol titration; but after exposure to pH 10 showed no activity of any sort on casein at pH 7.4. The consistent failure of normal gastric juice on casein at pH 7.4 to produce significant increases in amino nitrogen confirms the apparent absence of contamination with duodenal contents. This fact, together with the persistence of increase in total soluble nitrogen after exposure to pH 10,[9] suggests that trypsin and erepsin-like enzymes of gastric or duodenal origin were not responsible for the increases in total soluble nitrogen.

Clinical observations show that mixtures of beef muscle and gastric juice administered at pH 1.8 or 2.5 have no hematopoietic activity.[5] When pepsin was removed from gastric juice by exposure to pH 10 for 2 hours at 37.5°C.,[6] though the *in vitro* activity of the resulting gastric juice on casein at pH 7.4 was retained, there was no activity at pH 2.5. This is in agreement with the negative clinical result and also indicates the absence of pepsinogen in the gastric juice.

The above correlations suggest, but obviously do not constitute final proof, that the action on casein is due to intrinsic factor. They also do not necessarily imply that casein is a clinically effective extrinsic factor.

9311 P

Determination of Phenol Red in Gastric Contents.*

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A dilution indicator in gastric analysis is a substance employed for determining quantitatively the proportion of residual test meal present in a sample of gastric contents. Of the numerous substances so employed, phenolphthalein and phenol red (P.R.) are the most common. The former, however, is not suitable for the purpose because of its low solubility in water (Hollander, Penner, and Saltzman[1]), but the latter possesses most of the characteristics requisite

[9] Northrup, J. H., *J. Gen. Physiol.*, 1921-22, **4**, 261.

* This work was supported in part by a grant from the Friedsam Foundation.

[1] Hollander, F., Penner, A., and Saltzman, M., *Am. J. Dig. Dis. and Nutr.*, in press.

for an ideal dilution indicator (Gorham,[2] Bulger, *et al.*,[3] Wilhelmj, *et al.*[4]). A method for determining P.R. in the presence of bile and protein suspensions is described by Wilhelmj. His procedure is unsatisfactory because: (1) it involves a subjective color correction previous to a colorimetric determination, (2) in the presence of dark green bile even this correction is impossible, (3) it necessitates the preparation of a different standard of comparison for each bile-containing specimen, (4) it does not include a standardized technique for adding the (variable) volume of color-correcting solution to the colorimetric standard without introducing an indeterminate change in the P.R. concentration. Furthermore, no data are cited to indicate its quantitative reliability.

In order to eliminate these faults, we have developed a simple and reliable method for the simultaneous removal of bile pigment and protein without loss of P.R. by means of freshly precipitated $Zn(OH)_2$. Following preliminary centrifuging, 5 ml. of the P.R. solution are treated with finely powdered CaO, sufficient to neutralize the gastric HCl and make the solution distinctly alkaline; then one ml. each of NaOH (0.5 N) and $ZnSO_4$ (0.55 N) are added (the latter dropwise), mixed thoroughly, and centrifuged after standing for 15 minutes; finally, 5 ml. of the supernatant fluid are treated with 2 ml. of Na_3PO_4 (0.5 N) to remove excess Zn and simultaneously to adjust the pH to a value suitable for colorimetry. After centrifuging, the supernatant liquid is filtered with suction and compared in the colorimeter with a standard P.R. solution (the test meal itself) similarly treated. Color differences between unknown and standard were practically non-existent. Comparison of treated with untreated standard solutions showed that the Zn treatment causes no significant loss of P.R. We have already run several dozen determinations on P.R. solutions in egg albumin, gastric pouch secretion from dogs, and human stomach contents; the concentration of gastric fluid varied from 25% to 95% and of P.R. from 0.2 mg. to 4.0 mg. per 100 ml. of mixture. Each solution was prepared from a different specimen of gastric contents; at least half of these were thick with dark green bile. In practically every case observed and calculated results agreed to within 0.04 mg. per 100 ml.; this corresponds to 1% of the initial concentration of the indicator in the test meal, which is accurate enough for all clinical and experimental purposes.

2 Gorham, F. D., *J. A. M. A.*, 1923, **81**, 1738.

3 Bulger, H. A., Stroud, C. M., and Heideman, M. L., *J. Clin. Invest.*, 1928, **5**, 547.

4 Wilhelmj, C. M., Neigus, I., and Hill, F. C., *Am. J. Physiol.*, 1933, **106**, 381.

Concerning the pH to which the P.R. must be adjusted for colorimetry: Wilhelmj uses one ml. of saturated NaOH to 6.5 ml. of P.R. solution, which is equivalent to a final concentration of about 1.8 N NaOH. It is generally known, however, that P.R. will lose its color very rapidly in the presence of strong alkali (Thiel[5]) and we have found that this color loss may be appreciable even in one N NaOH (pH about 14) after one-half hour. In Wilhelmj's procedure, therefore, unless the solutions are alkalinized only a short time before being read and fresh portions of standard solution are prepared at short intervals, there is a great likelihood of error due to this progressive loss of color. On the other hand, we have found that at pH 11-12 no appreciable loss of color occurs, even after 4 hours; also, by colorimetric comparison of P.R. solutions at pH's 11 and 12 and by examination of the pH-dissociation curve (Clark,[6] p. 58), we have shown that the indicator attains its maximum degree of color in this same pH range. Since the pH of Na_3PO_4 solutions is likewise around 12, it follows that our P.R. solutions require no further alkali after the addition of excess phosphate for removal of residual Zn.

Finally, we have investigated the applicability of Beer's law in this situation. Using a standard solution of 1.0 mg. per 100 ml. (instead of the uppermost value of 4.0 mg.) we have found a direct proportionality between concentration and colorimeter reading throughout the entire concentration range of 4.0 to 0.2 mg. per 100 ml. In all cases, calculated and observed readings agreed to 1% or better. Thus, in spite of extensive variations in the scale reading, it is unnecessary to employ standards of different concentration for the upper and lower ranges of P.R. concentration in the unknowns. The scale reading corresponding to the standard solution may be 5.0, 10.0, 20.0 or 40.0 as conditions dictate.

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5 Thiel, A., *Monatsh. Chemie.*, 1929, **53**, 1008.

6 Clark, W. M., *The Determination of Hydrogen Ions*, 2nd ed., Baltimore, 1923.

9312

Effect of Anoxemia on the Impermeability of the Stomach to Water.

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It has been known for a long time that practically no water is absorbed from the normal stomach, but that the small and large intestine are responsible for this function.

In an attempt to explain this difference certain theories have been advanced. Fischer[1] postulates that water absorption is influenced by the affinity of the colloids for water, and that the colloids of the blood have the greatest affinity for water when the blood is relatively most venous. Since the blood of the colon is relatively more venous than that of the small intestine, and since the stomach is the least venous of all, Fischer believes that venosity is correlated positively with water absorption.

Koehler, *et al.*,[2] showed that anoxemia is fundamentally acidotic, and that the pH of the blood can reach 6.7 during severe anoxemia. The carbon dioxide combining power can also fall as low as 9.8 volumes percent. This work has been confirmed by Van Liere and his coworkers, and by other investigators.[3]

It was felt that a study should be made of absorption of water from a stomach whose blood supply is increased in venosity. Anoxemia is an agent by which a significant increase in the venosity of the blood can be brought about.

During the course of other studies on the effects of anoxemia on gastro-intestinal function, the opportunity was exercised to study the effect of anoxemia on the absorption of water from the stomach. Barbitalized dogs and cats (220 mg. sodium barbital per kilo, intravenously) were used. Two animals were chosen as near the same age and weight as possible. One served as a control, while the other was subjected to anoxemia.

The stomach was exposed by a mid-line incision and the lower end of the esophagus was ligated. The stomach was washed out

[1] Fischer, M. H., *The Physiology of Alimentation*, John Wiley and Sons, New York, 1907, p. 267.

[2] Koehler, A. E., Brunquist, E. H., and Loevenhart, A. S., *J. Biol. Chem.*, 1925, **64**, 313.

[3] Van Liere, E. J., David, N. A., and Lough, D. H., *Am. J. Physiol.*, 1936, **115**, 239.

with distilled water and the pylorus ligated. The ligatures were so placed that the isolated loop thus formed had virtually an intact blood supply. The stomach was then filled with a measured amount of distilled water at body temperature, taking care to avoid overdistension. The abdomen was closed and the animal kept warm.

The other animal was treated by an identical procedure, including the injection of exactly the same amount of water into the stomach. After the abdomen was closed this animal was placed in a respiratory chamber and was subjected to the desired degree of anoxemia. Partial pressures of oxygen of 117, 94, 80, 63, and 53 mm. of mercury were used. The normal pressure, of course, is 152 mm.

At the end of 3 hours the stomach was removed from each animal, slit open, and its contents carefully measured.

Results. Table I shows the amount of water absorbed in the normal animals and in those at the various degrees of anoxemia.

TABLE I.

Partial Pressures of Oxygen mms. of Hg	No. Animals	% Water Absorbed	Variation from Normal %
152	74	6.5	
117	4	5.0	—1.5
94	19	8.2	1.7
80	20	7.9	1.4
63	14	5.1	—1.4
53	17	6.2	—0.3
Aver.	74	6.5	0.0

The normal animals absorbed 6.5%, and the extremes of variation in the experimental animals were 5.0 and 8.2, respectively. The average of all the experimental animals without reference to the degree of anoxemia was 6.5%, identical with the figure for the control animals.

Our findings in the control animals agree with the accepted dictum that comparatively little water is normally absorbed from the stomach of anesthetized animals. Six and one-half percent absorption over a period of 3 hours is surely not significant, for normally water would not encounter an obstruction at the pylorus such as was produced here, but would readily pass into the small intestine.

The findings of Koehler, *et al.*,[2] certainly show that anoxemia produces a condition in which the blood supply to all of the organs is increased in relative venosity. Starling[4] states, moreover, that the small intestine, less venous than the colon, is more efficient in water absorption.

[4] Starling, E. H., *Principles of Human Physiology*, Lea and Febiger, Philadelphia, 1936, p. 560.

The factor of secretion into the stomach as an influence on the amount of absorption can be ruled out, for it has been shown[5] that there is normally very little secretion in response to filling the stomach with water, and that even very severe anoxemia has but little influence on the small amount of secretion which is present.

As a further check on the importance of the blood supply to gastric absorption the control part of the experiment was repeated in 11 freshly killed animals. The average amount of absorption in these animals was 6.3%, a figure closely comparable with the normal figure for living animals.

In view of these findings, and since very severe degrees of anoxemia failed to influence the amount of absorption by the stomach we feel that some other factor than the venosity of the blood supply must be of prime importance in the failure of the stomach to absorb water.

Summary. Barbitalized cats and dogs were subjected to various degrees of anoxemia, the most severe being a partial pressure of oxygen of 53 mm. of mercury. This causes a distinct increase in the venosity of the blood. It was found that no degree of anoxemia compatible with life could have any appreciable influence on the amount of absorption from the stomach. It was also found that about as much water is absorbed from the stomach of a dead animal as from that of a living one.

From our findings, and related evidence from other workers previously quoted, it is concluded that some other factor than the venosity of the blood is of chief importance in the failure of the stomach to absorb water.

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Hepatic Circulation Time in Unanesthetized Dogs.

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No report on the rate of blood flow through the liver in unanesthetized dogs has been found in the literature. Since we have had available a number of animals angiostomized according to the tech-

5 Sleeth, C. K., and Van Liere, E. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 208.

nique of London,[1] with cannulae on the hepatic and portal veins, we have determined circulation time by the cyanide method.

Our experience with the angiostomy technique has been discussed elsewhere.[2] We have had little difficulty in preparing the animals so that satisfactory injections could be made into the portal and hepatic veins. The time required for KCN injected into either vein to reach the carotid sinus was determined by recording respiration with a pneumograph strapped to the chest, the arrival of the cyanide at the carotid sinus being indicated by the first increase in respiratory excursion. The dogs, previously accustomed to lying quietly during the withdrawal of blood, were held gently on a table with the pneumograph in place until respiratory rate and amplitude were constant. The long needle, attached to a syringe containing the cyanide, was passed down the London cannulae into the vein and the respiration again watched for a short period. Making sure the animal was quiet, the KCN was injected and the time marked on the drum. The increase in respiration was usually abrupt after injection into the hepatic vein and gradual after portal injection. But in no case was it difficult to determine the onset of respiratory stimulation. Typical results are illustrated by Fig. 1.

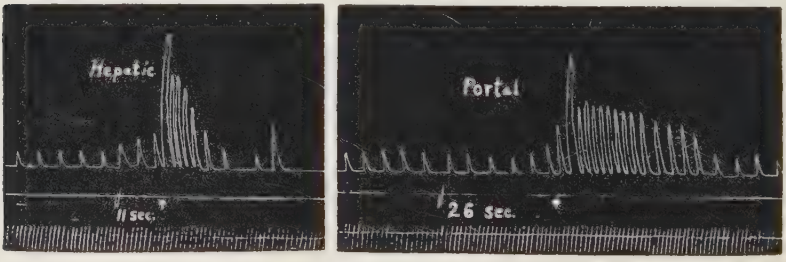

FIG. 1.
Injection of 1 mg. of KCN into hepatic vein, 3 mg. into portal. Normal unanesthetized dog. Respiration recorded.

The difference between the time required for respiratory stimulation after intra-portal injection and that required after intra-hepatic injection was taken as the hepatic circulation time. The results are summarized in Table I. It was necessary to inject from 3 to 6 mg. of KCN into the portal vein to obtain a response, as compared with one mg. in the hepatic. This may in part be due to distribution of the cyanide over a larger volume of blood by passage through the vascular bed of the liver, and in part to diffusion of the cyanide

[1] London, E. S., *Angiostomie u. Organestoffwechsel,* Moscow, 1935.
[2] Cherry, I. S., and Crandall, L. A., Jr., *Am. J. Physiol.* To be published.

TABLE I.

Hepatic Circulation Time in Unanesthetized Dogs.

H—Seconds elapsing between injection of 1 mg. KCN into hepatic vein and respiratory response.

P—Same for 3 to 6 mg. KCN into portal vein.

C.T.—Hepatic circulation time in seconds (P-H).

	Normal Dogs						Adrenal Denervated Dogs		
	1	2	3	3	4	5	1	2	3
P.	26	16	13	24	20	30	17	26	29
H.	8	11*	6	12	7	7	10	10	12
C.T.	18	5	7	12	13	23	7	16	17

	Treated Depancreatized Dogs					Hypophysectomized Dogs		
	1	1	2	2	3	1	2	3
P.	14	16	24	23	20	18	22	24
H.	6	7	11	10	8	8	10	12*
C.T.	8	9	13	13	12	10	12	12

*Injection made in leg vein instead of hepatic.

from the liver capillaries. It is also suggestive of a detoxifying action on the part of the liver. It is significant that 6 mg. of KCN were usually required when injected intra-portally in normal dogs, while respiration was as a rule stimulated by 3 mg. of KCN injected into the portal in depancreatized dogs. It is not improbable that there was some interference with liver function in the hypophysectomized and depancreatized animals, even though the latter were maintained on insulin and raw pancreas.

Since all values for hepatic circulation time in the operated animals are well within the limits of variation found in the normals it appears that adrenal denervation, pancreatectomy, or hypophysectomy did not appreciably change the rate of blood flow through the liver in these dogs. A conclusive statement concerning the effect of these surgical procedures should await determinations on a larger series of animals. But it may be pointed out that the average for the normal group is 13 seconds, that for the whole series 12 seconds.

The variations in rate of blood flow in the normal animals are comparable to the variations that we have observed[2] in fasting glucose output from the liver in such dogs, although no relationship between blood flow and glucose output can be assumed until parallel determinations are made.

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Innervation of the Intrinsic Muscles of the Eye: An Experimental Study.

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The innervation of the iris and the ciliary body of mammals has been well worked out physiologically, but the recent interest in the nerve fiber terminations in smooth muscle affords ample justification for further investigation of the nerves to the intrinsic muscles of the eye.

In an investigation of the endings on the smooth muscle of the gut, Stöhr[1] described the terminal structure as a reticulum or syncytium. Boeke[2] has described a rich supply to the ciliary muscle which resembles that described in smooth muscle by Stöhr. Using the Bielschowsky technic, Lawrentjew[3] has described degenerating fibers in the nictitating membrane after extirpation of the superior cervical sympathetic ganglion.

Our experiments have been carried out on cats. The Bielschowsky block technic and the protargol section technic have been used. The superior cervical and the ciliary ganglion were removed on the left side, both together in some cases and one or the other alone in others. The time allowed for degeneration varied from 2 to 52 days. The eye of the contralateral side was stained as a control.

In a series of cats sacrificed after degeneration periods of 7 and 14 days respectively in which both the superior cervical and the ciliary ganglion had been removed, no intact fibers could be found in the iris or ciliary body on the operated side. The contralateral side showed the normal picture.

In a group in which the ciliary ganglion alone was removed and the cats allowed to survive for 6 days, degenerating fibers could be demonstrated in the sphincter and ciliary muscles by the Bielschowsky method, but no intact fibers were observed.

The superior cervical ganglion of the left side was removed in another group and degeneration periods ranging from 7 to 52 days allowed. In none of these were any of the fibers of the fine plexus in the dilator pupillae muscle intact when stained by the protargol method. A few fibers could be seen running to the iris border where

[1] Stöhr, P., Jr., *Z. Zellforsch.*, 1932, **16**, 121; 1934, **21**, 243.

[2] Boeke, J., *Z. mikrosk.-anat. Forsch.*, 1933, **33**, 275; 1936, **39**, 477.

[3] Lawrentjew, B. I., *Z. Zellforsch.*, 1936, **23**, 560.

the dilator muscle is located, but none could be seen entering the muscle. The innervation of the ciliary muscle and of the sphincter appeared entirely normal.

In 4 cats, the ciliary ganglia were removed on the left side and the animals sacrificed at the end of 21, 16, 7, and 2-day periods. Stained by the protargol method, no trace of nerve fibers could be found in the ciliary body and sphincter of the iris on the operated side in animals which survived for 21- and 16-day periods. In the animal sacrificed on the seventh day, the sphincter and ciliary muscle contained but a few degenerated nerve fibers which were greatly swollen and varicose. In the eye for which the degeneration period was only 2 days, the finer fibers of the terminal plexus were present in part, but presented a granular and extremely varicose appearance. In all these cases the fine plexus in the dilator muscle remained intact.

My preparations of normal eyes stained with protargol agree in the main with those described by Boeke. The plexus is in much closer contact with the nerve fibers than is indicated in the illustrations of Stöhr. The fibers supplying the sphincter and ciliary muscles are of large calibre and a large percentage are myelinated. The fibers to the radial muscle are extremely fine. The axons leaving the more superficial plexus above the muscle ramify among the muscle fibers and give off many branches, usually at right angles, which run among the muscle fibers and show varicosities at various levels. In no case did the finer ramifications of the "terminal net" anastomose.

In sections cut transversely through the ciliary muscle at 4 and 6 microns, the nerve fibers can be seen to course in great numbers in close proximity to the muscle cells. They appear to become intraprotoplasmic in the more distal part of their course and show structures which appear as knoblike terminations near the nucleus. No ganglion cells were seen in either the chorioid, sclera, or uvea. No sensory endings were found in the smooth muscle and none were recognized in the stroma cells.

Summary. All nerve fibers in the ciliary and sphincter muscles degenerate following extirpation of the ciliary ganglion. All nerve fibers to the dilator pupillae degenerate after extirpation of the superior cervical ganglion. The terminal nervous structure in these muscles is not independent of the axons but undergoes degeneration with the latter following extirpation of their cells of origin. Terminal branches of the axons seem to enter the cytoplasm of the muscle cells.